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NITRATE REDUCTION COUPLED TO IRON(II) AND MANGANESE(II)
OXIDATION IN AN AGRICULTURAL SOIL

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in
Plant and Soil Science in the College
of Agriculture at the University of Kentucky

By

Stephanie Marie Pyzola

Lexington, Kentucky

Director: Dr. Chris J. Matocha, Associate Professor of Plant and Soil Science

Lexington, Kentucky

2013

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ABSTRACT OF THESIS

NITRATE REDUCTION COUPLED TO IRON(II) AND MANGANESE(II) OXIDATION IN AN AGRICULTURAL SOIL

New evidence shows iron(II) oxidation is strongly coupled to nitrate reduction under anaerobic conditions in freshwater sediments and agricultural soils. However, the contribution of iron(II) oxidation to nitrate reduction is unknown. Furthermore, oxidation of manganese(II) by nitrate has been largely overlooked. This study investigated nitrate-dependent iron(II) and manganese(II) oxidation in an agricultural soil (Sadler silt loam) using stirred-batch kinetic techniques with native soil organic carbon (SOC) as the electron donor and included addition of amendments (hydrogen gas and wheat residue). In the presence of native SOC, nitrate-dependent Fe(II) and Mn(II) oxidation occurred at early stages of the reaction while organic carbon participated at longer times. Contributions of iron(II) and manganese(II) oxidation to nitrate reduction were 19% and 25%, respectively. This is significant in light of excess SOC relative to total Fe and Mn in the Sadler soil. Addition of hydrogen gas lowered the contribution of iron(II) oxidation to nitrate reduction to 10%, while addition of plant residue raised this value to approximately 55%. Manganese(II) oxidation contributed 50% to nitrate reduction under hydrogen amended conditions. These coupled processes involving Fe(II) and Mn(II) oxidation are an underappreciated aspect of the nitrogen cycle and merit consideration in future studies.

Keywords: Nitrate Reduction, Iron(II) Oxidation, Manganese(II) Oxidation, Anaerobic Cycling, Redox Potential

Stephanie Marie Pyzola

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March 06, 2013

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OXIDATION IN AN AGRICULTURAL SOIL

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For my Granny, Emma Gene Capps

ACKNOWLEDGEMENTS

Writing this thesis has been a significantly challenging experience and was not possible without the help and encouragement of many. First, I owe a great deal of gratitude to my advisor, Dr. Chris Matocha, whose expertise, approachability, and excited personality made him an outstanding advisor and confidant. Secondly, I would like to thank Dr. Coyne and Dr. Grove for being members on my committee. Learning from them in and out of the classroom has truly been an exceptional privilege. Also, I want to thank Martin Vandiviere for all his patience with my frequent questions, as well as his help, great music, and good conversation in the lab. I also owe thanks to everyone in the labs of Dr. Coyne, Dr. McCulley, Dr. McNear, and Dr. Grove. I have appreciated all of their kind assistance and the ability to use their equipment.

I would also like to give my love and appreciation to my family: Mom, Dad, Sarah, and Tanner. Their love, support, and motivation were unwavering throughout this whole process. It will never go unnoticed and I cherish it deeply. Furthermore, I thank my closest friends and colleagues and UK Basketball for making the stress of Graduate School more bearable. Finally, I'd like to thank God for helping me get through Graduate School. Without Him, none of this would have been possible: "If God brings you to it, He will bring you through it".

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CHAPTER 1: INTRODUCTION

NITROGEN CYCLE

Nitrogen is the most abundant element in the Earth's atmosphere and is also present in soil and plants. Most atmospheric nitrogen is in the form of N_2 gas. The N_2 form of nitrogen is just one of the many chemical forms in which nitrogen can occur. The nitrogen cycle contains organic and inorganic forms of nitrogen. Organic forms of soil nitrogen occur as intricate compounds, including amino acids and proteins. Inorganic soil nitrogen can be in the form of nitrate, nitrite, ammonium, nitrous oxide, and elemental nitrogen (N_2), among others. Depending on the form of nitrogen in the soil, there are several different transformations that can occur, which make up various steps in the nitrogen cycle. These "steps" could include immobilization or mineralization, volatilization, leaching, plant uptake, fixation, and nitrification or denitrification (Figure 1.1).

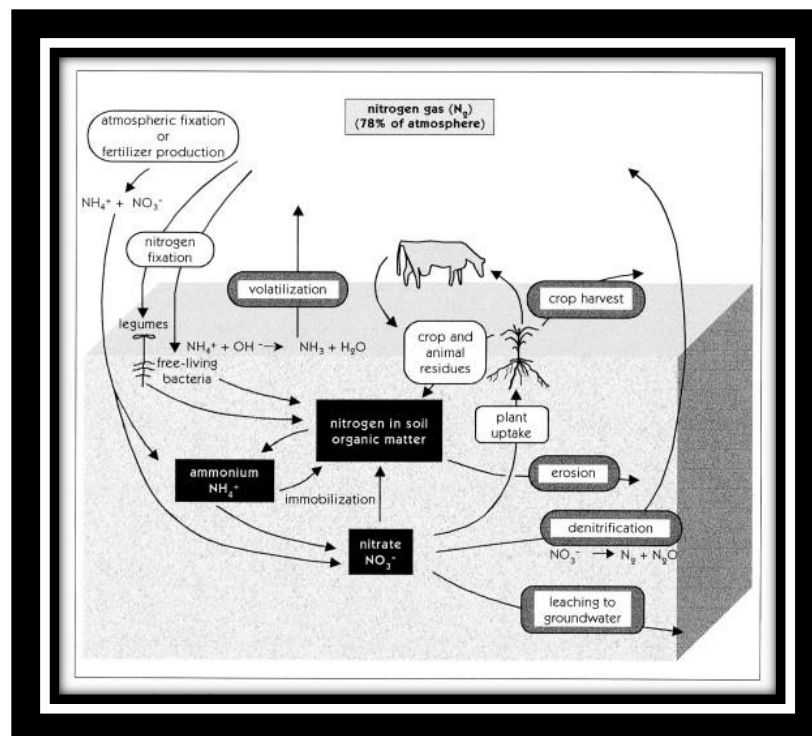


Figure source: NSW HSC Online

Figure 1.1: General overview of the nitrogen cycle.

The nitrogen cycle plays an important role in the soil environment, causing conversions of non-plant available nitrogen to plant available forms of nitrogen. Nitrogen cycling is also important to the soil environment because cycling allows inorganic forms of nitrogen to be transformed into organic forms and vice versa. Two important processes in the nitrogen cycle are nitrification and denitrification. Nitrification allows ammonia/ammonium to be oxidized to nitrate with nitrite as an intermediate. Oxygenated environments are important to this process. Denitrification is the process by which nitrate is reduced to dinitrogen, with possible intermediates being nitrite, nitric oxide, and nitrous oxide. This process generally occurs in wet, anaerobic environments in which available oxygen is not present and organisms use available nitrate and nitrite as electron acceptors, which results in the release of nitrous oxide and/or dinitrogen.

Nitrate Reduction

In the United States, excess and continual agricultural nitrogen fertilizer inputs have led to nitrate contamination of many ground and surface waters. High levels of nitrate result in negative environmental inputs such as eutrophication and human health issues such as Methemoglobinemia (blue baby syndrome) (Santamaria, 2006). Therefore, the removal of excess nitrate from soil and water is of great interest. One form of nitrate removal from soil and water is via the reduction of nitrate to gaseous nitrogen compounds. Nitrate reduction has been largely attributed to microbial activity although abiotic nitrate reduction has also been documented (Hansen, et al., 1996). Heterotrophic and autotrophic bacterial denitrification reduces nitrate in the following progression:



This process is associated with water saturated or near-saturated anaerobic soil conditions in which oxygen has been depleted from the environment (Smith and Tiedje, 1979). Abiotically, solution studies have shown that inorganic reduction of nitrate can occur with ferrous iron (Buresh and Moraghan, 1976), Fe(II) silicate minerals (Postma, 1990), Fe(II)-Fe(III) hydroxides known as green rusts (Hansen et al., 1996), and the Fe(II) mineral wüstite (Rakshit et al., 2005).

The oxidized form of nitrogen is nitrate, in which the charge on the oxidized N is +5. As nitrate is transformed during denitrification, the oxidation state of the N atom is reduced. Nitrate is especially prone to transformation because it is an extremely mobile

monovalent anion (Vitousek et al., 2002). During denitrification, nitrate (+5 oxidation state) can be progressively reduced to nitrite (+3), nitric oxide (+2), nitrous oxide (+1) and finally dinitrogen gas (0). The form that nitrogen takes in soil environments is greatly influenced by the redox potential of the system. In more oxidized or aerobic environments, nitrate is one of the dominant species present. For denitrification to become active, redox potential values below 420 mV must be achieved (Nikolaeva and Eremina, 2005). However, between 340-480 mV, nitrate and nitrite can coexist (Nikolaeva and Eremina, 2005). Gaseous nitrogen (NO, N₂O, N₂) tends to predominate at Eh values less than 200 mV (Nikolaeva and Eremina, 2005). Because pH also influences redox potential, the pH of a system can contribute to the Eh ranges in which transformations of nitrate occur. According to research by Patrick and Jugsujinda (1992), at pH 6.5 the redox potential at which nitrate fully disappears from soil solution due to reduction is 200-250 mV.

Coupling Nitrogen and Carbon

Historically, denitrification has been strongly associated with soil carbon, with various soil carbon sources serving as the electron donors under anaerobic conditions; more specifically dissolved organic and soil organic carbon (Burford and Bremner, 1975).

Equation 1 shows this association:



Burford and Bremner's research showed that nitrate reduction in soils under anaerobic conditions is controlled by the presence of readily decomposable organic matter. More specifically, they showed that increases in mineralizable and water-soluble organic carbon are the organic carbon sources most strongly associated with increased denitrification capacity.

Heterotrophic denitrifying microorganisms are assumed to be responsible for these processes because they obtain energy via coupling with organic carbon oxidation (Brady and Weil, 2008) as nitrate is reduced. The available carbon sources that denitrifying bacteria can utilize are vast and include acetate, ethanol, and glucose (Beauchamp et al., 1989; Muehe et al., 2009). Glucose, one of the main carbon sources for denitrifying bacteria, can reduce the redox potential to less than -300mV when added to anaerobic soil (Beauchamp et al., 1989). Manure has also been studied as a carbon source for denitrification but no substantial conclusions have been made regarding its ability to act as a readily available source of carbon

(Beauchamp et al., 1989). Plant residue was also noted as having potential to be an important stimulant of denitrification (Beauchamp et al., 1989), via increased available carbon levels for denitrifying organisms.

IRON CHEMISTRY

Iron is the fourth most abundant element on the Earth's surface (Havlin et al., 2005) and can have a range of oxidation states with the most common being Fe^{+2} and Fe^{+3} . Iron is present in many forms, including primary/secondary minerals such as hematite (Fe_2O_3), goethite (FeOOH), and magnetite (Fe_3O_4) (Havlin et al., 2005). Iron(III) can also be present in phyllosilicate minerals (Thamdrup, 2000). Iron (II) is most reactive as a reductant in complexed forms, such as solid Fe(II) minerals or adsorbed Fe(II) surface species (Rakshit et. al., 2005; Matocha, 2005). Water-soluble (dissolved) Fe(II) is generally less reactive, but microorganisms can readily utilize it as an electron donor (Straub et al., 1996). Ferrous iron tends to be more stable and resist oxidation by molecular oxygen or microbes when pH is acidic (Temple and Colmer, 1951).

Redox Chemistry Effects on Iron Oxidation State

Oxygen is the first species to act as prominent electron acceptor during organic matter decomposition, followed by nitrate, manganese (III, IV) minerals, iron (III) minerals, then sulfate, and finally carbon dioxide (Patrick and Jugsujinda, 1992). There is an Eh range in which each species is known to reduce. These Eh values, measured using a platinum electrode, serve as a guide, illustrating Eh conditions where transitions will occur. Redox potential changes in response to electron donors being consumed and electron acceptors being reduced. Redox potentials for the Fe oxides are variable and have been shown to vary between -300 and 0mV (Thamdrup, 2000) or at levels around 100mV (Patrick and Jugsujinda, 1992). Under aerobic or more positive redox potential conditions, iron is present in its oxidized state, Fe(III), while lower redox potentials are conditions in which the reduced form of iron, Fe(II), is present. Iron(III) will not be reduced when nitrate is present in soil (Patrick and Jugsujinda, 1992). An interesting component to iron redox chemistry is that the reduction potentials of the Fe(III) oxides increase by 59mV per unit decrease in pH allowing more energy to be available from iron reduction at greater pH (Thamdrup, 2000).

Iron Solubility

Microbial Fe(III) reduction in soil environments produces dissolved Fe(II), which is more soluble and plant available (Brady and Weil, 2008). At a redox potential of 100mV, Fe(II) begins to appear in solution (Patrick and Jugsujinda, 1992), however, this value only serves as a guide and is dependent on levels of poorly crystalline and well-crystalline Fe(III) minerals (Thamdrup, 2000). In addition, under anaerobic or reducing conditions, Fe(II) is much more soluble than its oxidized counterpart (Brady and Weil, 2008). For extracting soluble Fe(II) from a heterogeneous mixture such as soil, both water and acid extractions are valuable for distinctions between water soluble and sorbed species.

MANGANESE CHEMISTRY

Manganese is the twelfth most abundant element in the Earth's crust (Armstrong, 2008; Gerber et al., 2002). Manganese chemistry is complex, with oxidation states of Mn^{2+} , Mn^{3+} , or Mn^{4+} . Mn(II) is the dominant species in soil solutions, while all three oxidation states of manganese are present in soil minerals (Essington, 2004). Manganese(III) and Mn(IV) are found in assorted secondary minerals including pyrolusite (MnO_2), hausmannite (Mn_3O_4), manganite (MnOOH) (Havlin et al., 2005), and poorly crystalline minerals such as birnessite ($\delta\text{-MnO}_2$) (Essington, 2004). Solid-phase Mn(III, IV) oxides serve as good electron acceptors of diphenolic organic compounds and inorganic reductants as well (Matocha et al., 2001; Matocha, 2005) and are involved in processes such as anaerobic respiration (Learman et al., 2011), and have strong sorptive and oxidative capacity for various species (Learman et al., 2011).

Redox Effects on Manganese Oxidation State

The oxidized forms of manganese are Mn(III) and Mn(IV) present as oxide minerals whereas the reduced state is Mn(II). Mn-oxides are stable at higher Eh than Fe-oxides (Postma, 1985). These manganese oxides are readily reduced at fairly high redox potentials ($\sim 400\text{mV}$) (Nikolaeva and Eremina, 2005). Redox potentials for the Mn oxides have been shown to range between 500-600mV (Thamdrup, 2000). However, reduction predominates in a pH 6.5 solution, at an Eh value of approximately 200 mV, after all nitrate in the solution has been reduced, although overlap in the reduction of both Mn(III, IV) and nitrate has been reported (Patrick and Jugsujinda, 1992). Progression of redox potential on the

generalized redox ladder places manganese reduction after reduction of nitrate and preceding reduction of iron (Patrick and Jugsujinda, 1992). Nikolaeva and Eremina (2005) reported that wide ranges in Eh values corresponding with Mn(III, IV) reduction are due to the great diversity of Mn(III, IV) compounds in soil.

Redox Effects on Manganese Solubility

Oxidized forms of manganese have low solubility (Thamdrup, 2000) while the reduced species (Mn^{2+}) has high solubility (Sposito, 1989). The oxidized manganese species tend to precipitate as oxides, hydroxides, and oxyhydroxide minerals (Sposito, 1989). Manganese becomes increasingly soluble as the pH of soil solution drops (Brady and Weil, 2008). Reducing conditions will also increase manganese solubility (Havlin et al., 2005). At low pH and low Eh, approximately 100mV when manganese reduction has finished and iron reduction is beginning, Mn(II) is the dominant species present (Johansson, 2005) and is considered soluble (Patrick and Jugsujinda, 1992). When the pH and Eh are high, Mn(IV) is the dominant species present (Johansson, 2005). Mn(III) is readily oxidized to Mn(IV) when pH is low and there is a low concentration of Mn(II) present (Johansson, 2005). Although Mn(III) and Mn(IV) are particularly insoluble in water at neutral pH, solubility can be increased with chelation by organic ligands (Thamdrup, 2000). Under anoxic acidic and anoxic neutral environments, Mn(IV) oxides can chemically oxidize Fe(II) (Ratering and Schnell, 2001). Both water and acid extractions of soil mixtures demonstrate effectiveness in removing water soluble and sorbed manganese.

COUPLING NITRATE REDUCTION WITH IRON(II) OXIDATION

Early research on nitrate and iron showed that Fe(II) would result in the reduction of nitrate in the presence and absence of Cu(II), although greater denitrification occurred at higher levels of Cu(II) (Buresh and Moraghan, 1976). Buresh and Moraghan's research found that Cu(II) acted as a catalyst for Fe(II) reduction of nitrate to nitrite. Mineral forms of Fe(II) are more reactive towards nitrate than dissolved Fe(II). The mixed Fe(II)-Fe(III) mineral "green rust" can reduce nitrate to ammonium at significant rates while itself concurrently transforms to magnetite ($\text{Fe}_3\text{O}_4(\text{s})$) (Hansen et al., 1996). Reduction of nitrate has been shown to occur via the iron(II) oxide mineral wüstite (Rakshit et al., 2005). In this experiment, nitrate was added to iron(II) oxide, which rapidly consumed nitrate while

producing ammonium as the final nitrogen product of the reaction (Rakshit et al., 2005). Nitrite was present only as a transient intermediate and there was only negligible N_2O production (Rakshit et al., 2005). Nitrate reduction by detrital Fe(II) silicates has also been shown to occur, which resulted in small amounts of intermediate nitrite production, suggesting that the nitrate reduced to gaseous products (Postma, 1990). More recent research has demonstrated that nitrate reduction is strongly coupled with iron(II) oxidation (Weber et al., 2006; Matocha and Coyne, 2007; Muehe et al., 2009; Samarkin et al., 2010).

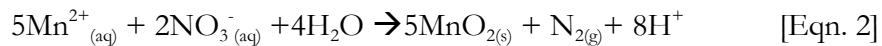
Chemical oxidation of iron by nitrate, nitrite, and nitrous oxide under anaerobic conditions, as well as biological oxidation by lithoautotrophs that use nitrate as the electron acceptor in the absence of oxygen, has been shown in many laboratory experiments (Straub et al., 1996). Biotically, various nitrate-reducing bacteria oxidize iron in freshwater sediments (Hauck et al., 2001). When nitrate is used as the electron acceptor in the absence of oxygen to oxidize iron(II), the process is called nitrate-dependent iron(II) oxidation. Under autotrophic growth conditions, nitrate dependent Fe(II) oxidation by a lithoautotrophic betaproteobacterium, Strain 2002, occurred and produced gaseous nitrogen products, N_2O and N_2 (Weber et al., 2006). Strains of denitrifying bacteria (LP-1, AR-1, and ToN1) have been enriched and grown anaerobically with nitrate and FeSO_4 , which resulted in the oxidation of Fe(II) (Straub et al., 1996). In the absence of nitrate in the media, Fe(II) was not oxidized, providing further evidence of nitrate-dependent iron(II) oxidation (Straub et al., 1996).

Addition of nitrate to flooded paddy soil where oxygen was not present resulted in iron(II) oxidation to iron(III) with concomitant nitrate reduction (Ratering and Schnell, 2001). This study was conducted *in situ* at varying soil depths, which confirms that this process is happening in natural agroecosystems (Ratering and Schnell, 2001). Furthermore, this research determined that mixotrophic nitrate-dependent iron(II) oxidizers were present at soil depths where nitrite existed as a nitrate reduction intermediate in concurrence with Fe(III), indicating microbes play an important role in nitrate dependent iron(II) oxidation in the soil environment (Ratering and Schnell, 2001). Nitrate-dependent iron(II) oxidation has also been reported in freshwater and marine sediments (Benz et al., 1998; Weber et al., 2006). This process has also been demonstrated in a moderately well drained agricultural soil (Matocha and Coyne 2007). In this latter study, native soil Fe(III) was not allowed to reduce

to Fe(II), so competitive processes (microbial Fe(III) reduction to Fe(II)) were operative during the reduction of nitrate (Matocha and Coyne, 2007). It is suspected that both biotic and abiotic activity play roles in nitrate-dependent iron(II) oxidation. While we know nitrate dependent iron(II) oxidation is occurring, the specific contribution of Fe(II) oxidation to nitrate reduction has not been clearly established, therefore more research in this area is needed.

COUPLING THE NITROGEN CYCLE WITH MANGANESE

Denitrification by microorganisms has long been considered the main method of nitrate transformation to reduced species. However, the possibility of nitrate reduction with the aid of Mn(II) has been documented (Aller, 1990; Luther et al., 1997). Aller (1990) documented NO_3^- and Mn(II) patterns in Panama Basin sediment pore water at increasing depths. This data showed that after depletion of nitrate, there was an accumulation of Mn(II) which decreased when nitrate levels began to accumulate after depths of 12cm. From this, Aller (1990) suggested an idealized pore water reaction in which nitrate reduction to N_2 and manganese oxidation take place concurrently, shown by Equation 2 below:



Similar findings were found in South Atlantic sediments in which the reoxidation rate of Mn(II) significantly affected nitrate reduction rates (Schulz et al., 1994). Schulz et al. (1994) determined that this is evidence of reoxidation of Mn(II) by nitrate, which could indicate manganese plays a potentially important role in nitrogen reduction. More recently, in research in anaerobic conditions in sedimentary zones, NO_3^- was reduced to N_2 by Mn(II) (Luther et al., 1997). Tebo (1991) monitored manganese (II) oxidation in anoxic conditions in the Black Sea where he saw a disappearance of manganese, purportedly due to Mn(II) adsorption or possibly Mn(II) oxidation by nitrate via the involvement of microbes (Luther et al., 1997). Work in pore water of deep-sea sediments has also shown that nitrate may act as a model oxidant for Mn(II) when oxygen is not present (Luther et al., 1997). The trend for nitrate acting as an oxidant of Mn(II) when oxygen is not present has been suggested in many studies (Aller, 1990; Luther et al., 1997; Schulz et al., 1994). Unfortunately, contributions of Mn(II) oxidation to nitrate reduction have not been established.

COUPLING IRON(II) OXIDATION WITH MANGANESE (III, IV) REDUCTION

As mentioned in the previous section, nitrate reduction has been closely associated with Fe(II) oxidation. Another potential sink for Fe(II) removal is oxidation by Mn(III, IV) oxide minerals (Thamdrup, 2000). Anoxic reactions between the Mn-oxide mineral birnessite and Fe(II) resulted in the production of Mn(II) via Fe(II) oxidation to Fe(III) (Postma, 1985). In Postma's (1985) experiment, if the pH was below 4, the reduction of birnessite was very quick; however, when pH was at or greater than 4, the birnessite and Fe(II) reaction was slower. The release of the Mn(II) was slowed at higher pH, changing the reaction to release Fe(III) and subsequently precipitate FeOOH (Postma, 1985). Furthermore, the slower release of Mn(II) was expected to be due to FeOOH precipitation directly on the birnessite surface, therefore blocking the reactive sites, indicating that the surface reactions determine the reaction rate (Postma, 1985). Similar findings were found by Postma and Appelo (2000) in a column flow system. Because Mn(II) and Fe(II) have the affinity to readsorb to their own oxides (Thamdrup, 2000), it is possible that Fe(II) could sorb to Mn-oxide surfaces, resulting in electron transfer and ultimately in the oxidation of Fe(II). This secondary reaction might be important where there are unreduced Mn(III, IV) oxide minerals that oxidize released Fe(II) (Lovley and Phillips, 1988), or where Mn(II) is oxidized by nitrate, and the resulting Mn(III, IV)-oxides could oxidize Fe(II). This latter scenario is occurring in our experiments (see chapter 2) and could interfere with the analysis of Fe(II) oxidation's contribution to nitrate reduction. Therefore, use of an Fe(II) binding agent (i.e. ferrozine) may inhibit this secondary reaction to examine the contribution of only Mn(II) oxidation to nitrate reduction. Research evaluating the contribution of Mn(II) oxidation to nitrate reduction is lacking, therefore continued work in this area, and in soil media as opposed to water or sediment, is merited.

OBJECTIVES

The objectives of this thesis are to:

1. Determine the contribution of Fe(II) and Mn(II) oxidation to nitrate reduction in agricultural soil slurries of the Sadler silt loam by a comprehensive wet chemical analysis (Chapter 2).

2. Evaluate the effect of an electron donor (H_2) and winter wheat residue on nitrate-dependent Fe(II) and Mn(II) oxidation (Chapter 3).

ORGANIZATION OF THESIS

Chapter 1 provides background information, an overview of the research problem, and objectives. Chapters 2 and 3 provide a detailed description of work done to satisfy the objectives of the thesis. Chapter 4 discusses conclusions of the research.

CHAPTER 2: NITRATE DEPENDENT IRON(II) AND MANGANESE(II) OXIDATION

Historically, carbon has been considered the most important electron donor for nitrate reduction (Burford and Bremner, 1975). Nitrate reducers can utilize various carbon sources for the reduction process. Experiments have shown nitrate reduction with the addition of plant residues (Paul and Beauchamp, 1989), while others show nitrate reduction with the addition of lower molecular weight carbon sources (Burford and Bremner, 1975). Some of these low molecular weight carbon sources include glucose, mannitol, sucrose (Burford and Bremner, 1975), and acetate (Chidthaisong and Conrad, 2000). Nitrate reducers were shown to utilize acetate in the presence of nitrate in a rice field soil, resulting in production of carbon dioxide (Chidthaisong and Conrad, 2000). In pure culture studies, the rate of nitrate reduction was greatest where acetate was utilized as an electron donor and carbon source (Van Rijn et al., 1996). A recent comparison of growth yield determinations using pure cultures of denitrifying bacteria showed that acetate resulted in greater yields when compared with formate (Strohm et al., 2007).

Other elements have also been shown to play a role in nitrate reduction. Recently, Fe(II) has been shown to serve as the electron donor for nitrate reduction. Nitrate-dependent iron oxidation has been reported in sediments, agricultural soils, deep-water zones, and in flooded paddy soil (Ratering and Schnell, 2001; Weber et al., 2006; Matocha and Coyne, 2007; Muehe et al., 2009; Samarkin et al., 2010). This process is important in subsurface environments as the first step in nitrate reduction is primarily biological (Roden, 2012) unless Fe(II) minerals such as green rust or wüstite are present, which can abiotically reduce nitrate (Matocha et al., 2012). Most of the microorganisms involved are considered mixotrophs, oxidizing Fe(II) to gain energy but requiring the presence of an organic co-substrate such as acetate (Muehe et al., 2009; Pantke et al., 2012). There are a few instances where pure lithotrophic microorganisms have been identified (Roden, 2012). In fact, a pure lithotrophic culture originally described by Straub et al. (1996) can couple nitrate reduction to water-soluble Fe(II) and mineral Fe(II) oxidation (Weber et al., 2001; Shelobolina et al., 2012) utilizing only inorganic carbon as a C source.

While nitrate-dependent Fe(II) oxidation has been shown, the contribution of Fe(II) oxidation to nitrate reduction has not been well documented. This is due in part to previous

studies in which Fe(II) oxidation occurred during the transition period between anaerobic and aerobic stages (Matocha and Coyne, 2007). If microbial Fe(III) reduction is still occurring at the time of nitrate addition, it confounds the calculation of moles of Fe(II) oxidized compared with nitrate reduced because Fe(II) is being produced concomitantly.

Manganese is often overlooked in association with nitrate reduction because of its lack of abundance in the Earth's crust. However, Mn(II) may be an additional electron donor for nitrate reduction. In deep-sea sediments, Luther et al. (1997) speculated that Mn^{2+} oxidation was coupled to nitrate reduction. This process might account for patterns in Mn(II) oxidation in two other studies (Tebo, 1991; Oguz et al., 2001). Upon closer inspection of the literature, it has been reported that NO_3^- immediately inhibited Mn(IV) reduction to Mn(II) by 50% when added to a pure culture of *Shewanella*, a well-known Mn(IV)-reducing microorganism (Myers and Nealson, 1988). In soil slurries where both iron and manganese are present, if Mn(II) is oxidized by NO_3^- to form Mn(III, IV)-oxide minerals, Fe(II) has the potential to adsorb to Mn(III, IV)-oxides (Canfield et al., 1993), so oxidation of Fe(II) by Mn(III, IV)-oxides could occur and act as a secondary reaction competing with the oxidation of Fe(II) by nitrate. If this secondary reaction occurs, the contribution of Fe(II) oxidation to nitrate reduction would be unknown. Therefore, the contribution of Mn(II) oxidation to nitrate reduction would need to be accounted for in order to establish an accurate contribution of Fe(II) oxidation to nitrate reduction. To account for only the contributions of Mn(II) oxidation to nitrate reduction, ferrozine can be used as an Fe(II) complexing agent to eliminate the secondary reaction of Mn-oxide induced Fe(II) oxidation. Elimination of this secondary reaction using ferrozine has been used in other studies to assess contributions of organic carbon oxidation to various terminal electron acceptors such as Mn(IV) and Fe(III) oxides (Canfield et al., 1993). Ferrozine is a good ligand for chelation and has been shown in many studies to not interfere or complex with manganese (Chapin et al., 2002; Sarradin et al., 2005; Stookey, 1970), making it an excellent binding agent for this study. Interference studies by Chapin et al. (2002) showed ferrozine effectiveness was not compromised by Mn(II) concentrations, up to 1000 μM levels.

The objective of these experiments is to determine the contribution of both Fe(II) and Mn(II) oxidation to nitrate reduction in agricultural soil slurries of the Sadler silt loam via comprehensive wet chemical analysis.

MATERIALS AND METHODS

Iron(II) Oxidation Contribution to Nitrate Reduction

This method is similar to the chemical analysis of stirred-batch method of Matocha and Coyne (2007) with the use of hydrochloric acid extraction, rather than oxalate extraction, to follow changes in Fe and Mn chemistry. Another difference was soil slurries were allowed to reduce all microbially reducible Fe(III) to Fe(II) prior to nitrate addition. Anaerobic conditions were important in this experiment to prevent oxygen from reacting with Fe(II). All sampling and reactive work for these experiments was done in an Argon purged anaerobic chamber (Coy Laboratory Products, Grass Lake, MI). All solutions used in the experiments were prepared in the glovebox with deionized water, which was deoxygenated with Ar gas for 3 hours before transferring into the glovebox. A Clark-type polarographic electrode (Warner Instruments, Hamden, CT) was used to measure oxygen content in the deionized water to ensure deoxygenation.

To begin, 14 grams of <2mm sieved Sadler soil (Oxyaquic Fraglossudalf, moderately well drained, silt loam soil) was placed into a 160mL glass serum bottle with a stir bar in duplicate. These bottles were transferred into the glovebox where a volume of 140mL of deoxygenated water was added. The time at which water was added to the soil was recorded as time zero. These bottles were sealed with a rubber septum and aluminum cap using a crimper. Both bottles were removed from the glovebox and placed on a shaker at low speed. At the following time points: 24 hours, 48 hours, 72 hours, 7 days, 14 days, 21 days, and 28 days following initial water addition, the bottle containing 140mL-deoxygenated water was taken back into the glovebox for sampling. Inside the glovebox, the bottle was uncapped and placed on a magnetic stir plate set at 300 rpm to allow for uniform mixing of the suspension. At the chosen time points previously described, soil pH and Eh were recorded, 0.5mL soil solution was removed and treated with 0.67M HCl for 1 hour using a rotisserie, and 7mL of the soil solution was extracted and filtered using 0.2 μ m Fisherbrand filter paper. One milliliter of the filtrate was complexed with 0.01M ferrozine [3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4], 0.1M MES buffer, pH 6, and deoxygenated water. After the HCl treated soil slurry finished its one-hour incubation, the solution was filtered and complexed with 0.1M MES buffer pH 6 and .01M ferrozine. This solution received a one-hour period to allow for color development before analyzing the sample via ultraviolet-visible

spectroscopy (UV-VIS). Both the complexed samples (water and HCl treated) were analyzed on the UV-VIS (double beam Shimadzu UV-3101PC spectrophotometer) at 562nm to determine absorbance of the Fe(II)-ferrozine complex (Stookey, 1970). To determine anion concentrations using ion chromatography, filtrates were run on a Metrohm 800 series modular IC with a Metrosep 250/4.0 and MetroSep RP guard disc holder and 3.6mM Na₂CO₃ eluent (Metrohm, Houston, TX). Additional tests performed on the complexed, water and acid extracted samples, were total Mn and total Fe absorption using flame atomic absorption spectroscopy (FAAS) (Shimadzu AA-6800, Kyoto, Japan). Ammonium concentrations in water filtrates were analyzed colorimetrically (modified indophenol-blue (Ngo et al., 1982)) using a plate-reader. Water filtrates were also subjected to total organic carbon analysis (Shimadzu, Kyoto, Japan).

Parallel 160mL glass serum bottles were prepared in the same manner at identical solid:solution ratios (10 grams soil:100mL water) to follow headspace gas characteristics via gas chromatography (GC). Nitrous oxide was measured using a Shimadzu GC-8A gas chromatograph fitted with a ⁶³Ni electron capture detector at 270°C and a Porapak Q column (Alltech Associates, Inc., USA) (40°C) using nitrogen as the carrier gas (30 mL min⁻¹). Carbon dioxide was measured using a Shimadzu GC-8A gas chromatograph fitted with a thermal conductivity detector operated at 100°C and a Porapak Q column (Alltech Company) (50°C) using helium as the carrier gas (30 mL min⁻¹). Analysis of the gases was complete within five minutes of injection.

On day 28 of sampling, 0.1mM nitrate was added to both serum bottles in anoxic conditions. This was taken as the new time 0 and following that, sample times were at 5 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 24 hours, 48 hours, and 72 hours. For each post-nitrate addition sample, the filtration, complexation and analysis, and the headspace gas analysis, was the same as the incubation sampling and analysis outlined above.

Treatments were duplicated.

Manganese(II) Oxidation Contribution to Nitrate Reduction

Methods are similar to those in the previous section. The only difference was on day 28 of sampling, 1mM ferrozine was added to each stirred-batch experiment. Ferrozine was allowed to react with the soil slurries for 10 minutes. Given that the reaction of ferrozine with Fe(II) is very rapid in pure solutions (Thompson and Mottola, 1984), this was deemed

sufficient time for all the Fe(II) to be chelated in the Sadler soil solutions. After this point, 0.1mM nitrate was added to both serum bottles. This was taken as the new time 0 and following that, sample times were at 5 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 24 hours, 48 hours, and 72 hours. For each post-nitrate addition sample, the filtration, complexation and analysis, as well as the headspace gas analysis, were the same as the incubation sampling and analysis outlined previously. Treatments were duplicated.

RESULTS AND DISCUSSION

The Sadler soil is a silt loam that is moderately well drained with an initial pH of 7.1 and is composed of 22% sand, 67% silt, and 11% clay. Table 2.1 shows the chemical characteristics of the Sadler silt loam soil. Various fractions of extractable iron and manganese are presented in Table 2.1 for unreduced Sadler, whole soil and the clay fraction. Oxalate- to dithionite-extractable Fe ratios were 0.39 and 0.38 for the whole soil and clay fraction, which suggests the presence of well-crystalline Fe oxide minerals (Schwertmann and Cornell, 1991). Past studies note that phyllosilicate Fe(III) is also present (Matocha and Coyne, 2007; Matocha et al., 2012). Total Fe was roughly 20-fold greater than total Mn in the Sadler whole soil (Table 2.1). Despite its lower total abundance relative to Fe, a greater fraction of manganese was extractable, relative to total Mn, than when compared with Fe. Dithionite extracted approximately 55% of the total Fe whereas 90% of the total Mn was extracted. Mineralogy of the clay fraction determined using x-ray diffraction showed the presence of hydroxyl-interlayered vermiculite, kaolinite, mica, and a trace of vermiculite. Total organic carbon (TOC) measured 13 g kg⁻¹ and total nitrogen measured 1.2 g kg⁻¹.

Table 2.1: Iron and Mn content of the unreduced Sadler silt loam; whole soil and clay fraction[†].

Extraction	Whole soil	Clay $\mu\text{mol g}^{-1}$
Fe_T	335.5 \pm 0.79	701.3 \pm 12
Fe_{CBD}	185.6 \pm 60	347.6 \pm 3.5
Fe_{AOD}	74 \pm 5.5	132.7 \pm 10.5
Fe_{HCl}	15.7 \pm 0.22	Not determined
Mn_T	15.92 \pm 2.3	4.61 \pm 0.01
Mn_{CBD}	14.4 \pm 0.7	3.92 \pm 0.01
Mn_{AOD}	14.7 \pm 2.3	3.51 \pm 0.12
Mn_{HCl}	4.66 \pm 0.05	Not determined
Mn_{HA}	12.61 \pm 0.78	0.34 \pm 0.01

[†] Fe_{CBD} and Mn_{CBD} (citrate-bicarbonate-dithionite extractable Fe and Mn, respectively) were extracted according to Mehra and Jackson (1960); Fe_{AOD} and Mn_{AOD} extracted according to Lovley and Phillipps (1986). Fe_T and Mn_T extracted according to Bernas (1968). Fe_{HCl} and Mn_{HCl} extracted according to Lovley and Phillips (1986) and Fredrickson et al. (1998).

Iron(II) Oxidation Coupled to Nitrate Reduction

Preincubation

A preincubation was conducted to reduce native Fe(III) to Fe(II). In soil slurries, there are other naturally occurring terminal electron acceptors present, (nitrate, Mn(III, IV), and sulfate) and we followed these changes. Preincubation lasted twenty-eight days, during which the pH of the soil slurry rose slightly from 7.37 to an average of 7.55 while the Eh

dropped into reducing conditions (Figure 2.1). The redox potential dropped rapidly over the first 14 days, from almost 150mV to about -50mV, where it remained level for the remaining incubation period (Figure 2.1). The rise in pH may have been due to the reduction of native Mn(III, IV) and Fe(III) minerals, which has been documented to increase alkalinity in correlation with organic carbon degradation (Vile and Wieder, 1993).

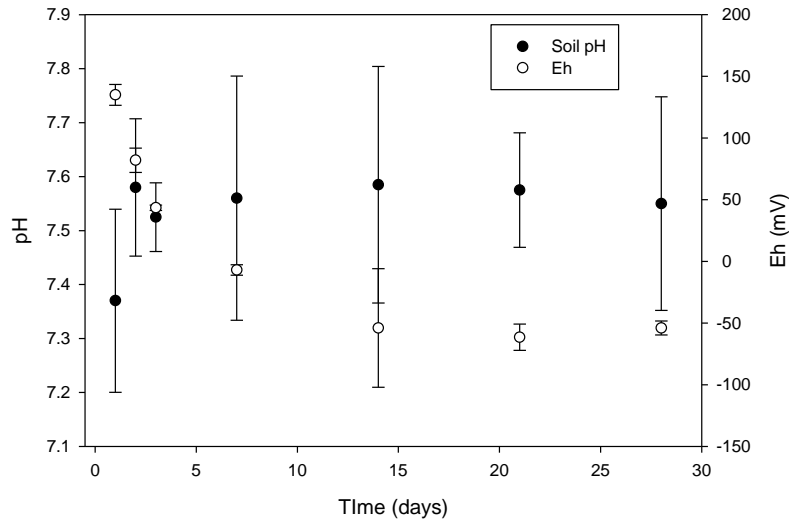


Figure 2.1: Soil pH and Eh during preincubation of the control experiment (error is standard deviation of mean data point).

The thermodynamic reduction sequence, based on decreasing redox potential, begins with O_2 , then NO_3^- , Mn(III, IV), Fe(III), SO_4^{2-} , and finally CO_2 (Acht nich et al., 1995). As Eh dropped in the anaerobic incubation, this is roughly the order in which reduction of the native species was predicted. Dissolved O_2 was not measured. However, the anaerobic preparation and incubation resulted in minimal O_2 within the soil slurries, which was probably consumed rapidly. Native nitrate concentrations were extremely low initially and then dropped to even lower concentrations, almost below detection limit, over the 28-day incubation. This early reduction of nitrate corresponds with the slurry's Eh being below 250mV, the redox potential previously mentioned as ideal for reduction or disappearance of nitrate, and the formation of gaseous nitrogen products (Patrick and Jugsujinda, 1992; Nikolaeva and Eremina, 2005).

After native nitrate was reduced, Mn(III, IV) reduction began. As the Eh became more negative, Mn(II) concentrations increased over the 28-day incubation (Figure 2.2), indicating that native Mn(III, IV) was being reduced. Reduction began quickly and was most

rapid between 24 hours and 7 days. Acid extractable Mn(II) concentration was initially much greater than the water extractable Mn(II) concentration, with water extractable Mn(II) leveling off at $2 \mu\text{mol g}^{-1}$, an approximately $1.75 \mu\text{mol g}^{-1}$ change in concentration. Acid extractable Mn(II) reached a plateau after 7 d, corresponding to the Mn_T (Table 2.1). This shows that all of the soil Mn is reducible under our experimental conditions, where native soil organic carbon is the sole electron donor.

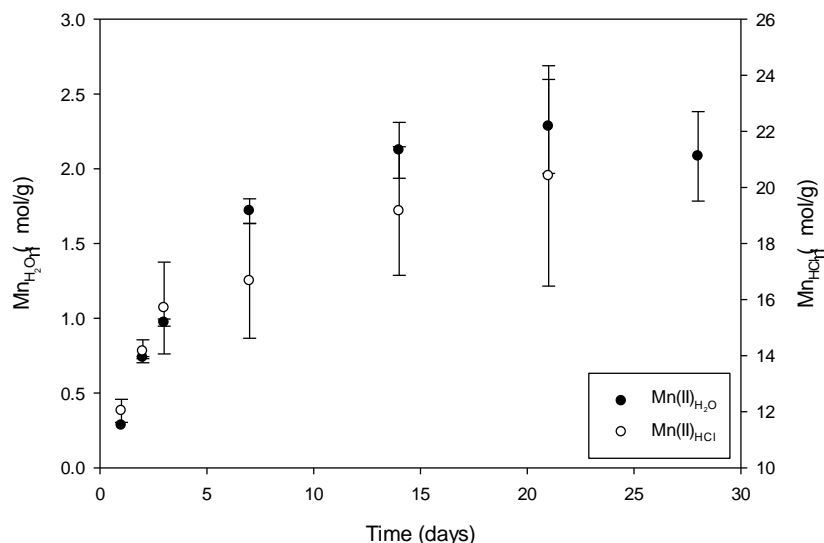


Figure 2.2: Water and acid extractable Mn(II) concentrations during the anaerobic preincubation (error is standard deviation of mean data point).

As the Eh dropped, there was an increase in both the water and HCl extractable Fe(II) concentration, indicating reduction of native Fe(III) to Fe(II) (Figure 2.3). This increase was rapid between 3 and 20 days, after which the Fe(II) concentration leveled off, suggesting the reduction of all native Fe(III) in the soil slurry. Full reduction of Fe(III) took place between day 21 and day 28, when the Eh was approximately -50mV, a redox potential lower than the 100mV previously documented for full Fe(III) reduction (Nikolaeva and Eremina, 2005). Fe(III) reduction is influenced by the presence of reactive organic matter (Thamdrup, 2000). Because native soil was used, and no OM was removed before incubation, the OM could be contributing to Fe(III) reduction during preincubation. A noteworthy feature of Figure 2.3 is the much greater concentration of HCl extractable Fe(II), shown on the secondary y-axis, than the water extractable Fe(II) concentration. The acid extractable Fe(II) concentration is 100x greater than the water extractable Fe(II)

concentration suggesting a greater amount of sorbed and precipitated Fe(II) as opposed to water-soluble Fe(II) in the native soil. This trend in extractable Fe(II) is consistent with past studies (Fredrickson et al., 1998; Matocha and Coyne, 2007).

In contrast to Mn, the plateau in acid extractable Fe(II) corresponded to approximately 12% of the total Fe ($40/335.5=0.119$). This indicates that a smaller fraction of Fe is microbially reducible. This is probably related to the crystalline nature of much of the Fe pool in the Sadler soil ($\text{Fe}_{\text{oxalate}}/\text{Fe}_{\text{dithionite}}=0.39$, Table 2.1) which is not as available to microorganisms as is poorly crystalline Fe (Thamdrup, 2000).

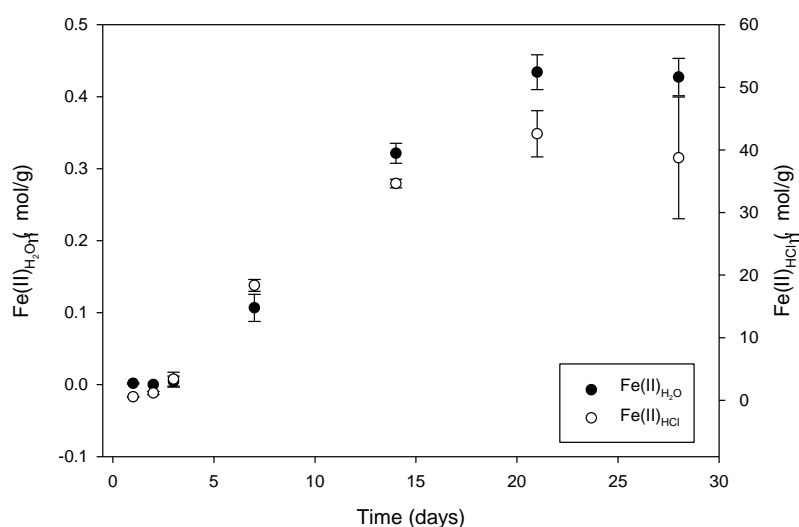


Figure 2.3: Water and acid extractable Fe(II) concentrations during anaerobic preincubation (error is standard deviation of mean data point).

Sulfate concentration increased slightly over the first week of incubation, but between day 7 and 14, there was a drastic drop in sulfate concentration in the slurry (Figure 2.4). This drop in sulfate concentration suggests that sulfate was reduced, most likely to sulfide. Other studies have noted a slight increase in sulfate levels followed by a decrease due to reduction (Achtnich et al., 1995).

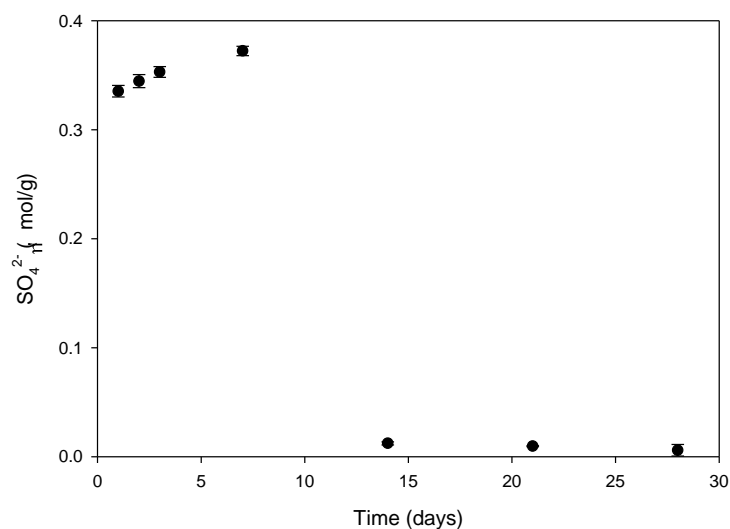


Figure 2.4: Sulfate concentration during anaerobic preincubation (error is standard deviation of mean data point).

A rapid rise in acetate concentration occurred over the 28-day preincubation period (Figure 2.5), and acetate leveled off at $\sim 8 \mu\text{mol g}^{-1}$. Anaerobic formation of acetate is most likely due to fermentation. Experiments by Chin and Conrad (1995) showed anaerobic accumulation of acetate in paddy soil during degradation of organic matter. They proposed that organic matter is first broken down via fermentation and then those products are broken down to acetate via homoacetogenic bacteria (Chin and Conrad, 1995). The Sadler soil was incubated in its native form, so biological activity may be playing an important role in this process. Specifically, proton-reducing bacteria may play a role in metabolizing fatty acids, resulting in an increase in acetate concentration (Chidthaisong and Conrad, 2000). The slight drop in acetate concentration after 28 days may be due to a reduction of acetate to ethanol (not measured) (Younesi et al., 2005). Younesi et al. (2005) showed that acetate production was mainly due to fermentation but the decrease in acetate concentration at longer times was due to acetate reduction to ethanol via the acetogenic bacteria pathway (Younesi et al., 2005).

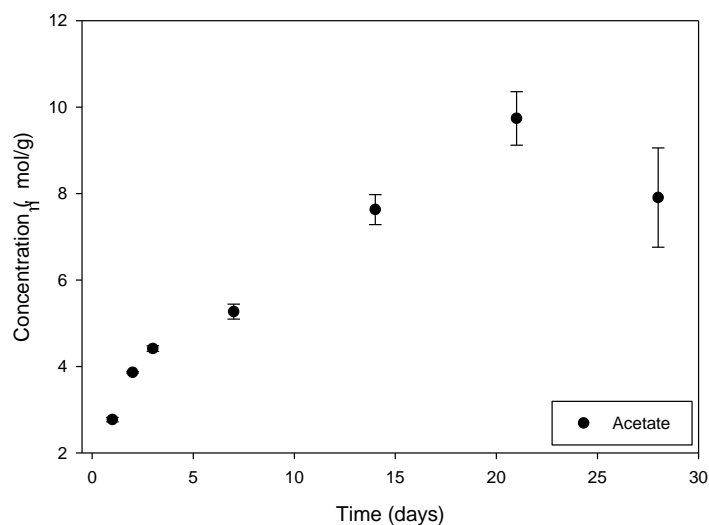


Figure 2.5: Concentration of acetate during anaerobic preincubation (error is standard deviation of mean data point).

Post Nitrate Addition

Immediately after the addition of an aliquot of 0.1M NaNO_3 , nitrate concentration decreased linearly over the first 6 hours at a rate of $0.027 \mu\text{mol g}^{-1} \text{ hour}^{-1}$ (Figure 2.6). This rate of nitrate reduction was similar to the nitrate reduction rate found by Achtnich et al. (1995), which was $0.84 \mu\text{mol g}^{-1} \text{ day}^{-1}$ or $.035 \mu\text{mol g}^{-1} \text{ hour}^{-1}$. After the first 6 hours, the nitrate concentration approached the detection limit and remained constant. While nitrate had nearly disappeared after six hours of reaction time, there was an appearance of nitrite at 24, 48, and 72 hours (Figure 2.6).

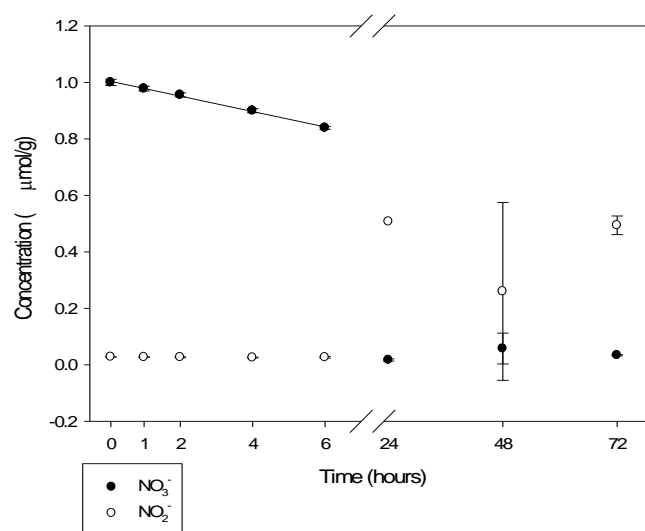


Figure 2.6: Nitrate and nitrite concentrations after the addition of NaNO₃ under anaerobic conditions. ($y = -0.027x + 1.01$; $R^2 = 0.997$; error is standard deviation of mean data point)

Along with the presence of nitrite, there was an increasing concentration of nitrous oxide until the 6-hour time point (Figure 2.7). A lack of ammonium formation (data not shown), and the appearance of both nitrite and nitrous oxide while the concentration of nitrate decreased, provides evidence that nitrate was reduced to nitrogenous gases. This anaerobic formation of nitrite and nitrous oxide, with no substantial production of ammonium, is similar to results found by Weber et al. (2006) and Luther et al. (1997).

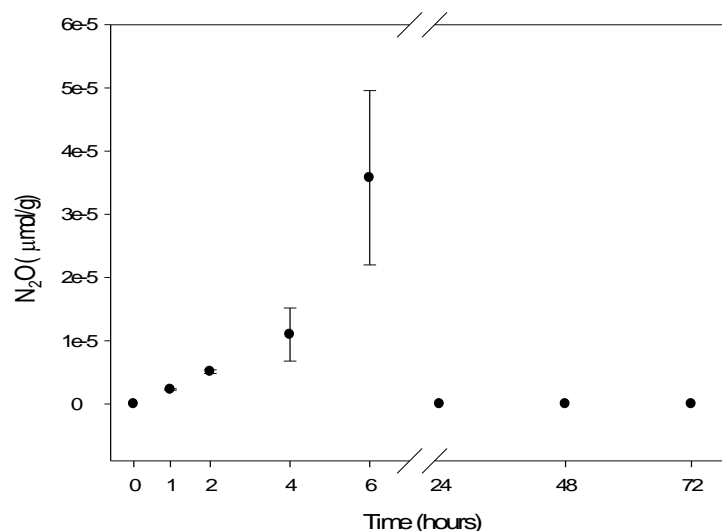
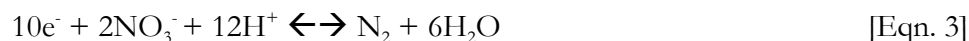


Figure 2.7: Nitrous oxide concentrations after the addition of NaNO_3 under anaerobic conditions (error is standard deviation of mean data point).

Post nitrate addition, a gradual increase in Eh occurred, rising to the original Eh of almost 150mV (Figure 2.8) while the pH increased from 7.6 to 7.9. This rise in Eh provides evidence that the addition of nitrate stimulated reestablishment of oxidative conditions in the soil slurry. Furthermore, there is a small rise in pH (Figure 2.8). Although the error over the first six hours is sizeable, if this increase in pH is real, it may have been caused by reduction of nitrate, which decreases the concentration of hydrogen ions in the slurry according to Equation 3 shown below.



There was an immediate drop in water-soluble and HCl extractable Mn(II) when nitrate was added during the first 6 hours of reaction (Figure 2.9). The E_h values (-50 to 0 mV) during this time frame (Figure 2.8) were well below the E_h levels associated with NO_3^- , Mn(IV), and Fe(III) reduction, as reported by Patrick and Jugsujinda (1992). This indicates that the Mn(II) oxidation coupled to nitrate reduction was not due to changes in E_h brought about by nitrate addition. The water and HCl extractable Mn(II) concentrations disappeared at rates of $0.178\mu\text{mol g}^{-1} \text{ hour}^{-1}$ and $0.630\mu\text{mol g}^{-1} \text{ hour}^{-1}$, respectively during the first 6 hours. At longer times (24 h and beyond), Mn(II) oxidation decreased, commensurate with nitrate depletion and higher E_h values (Figures 2.6 and 2.8).

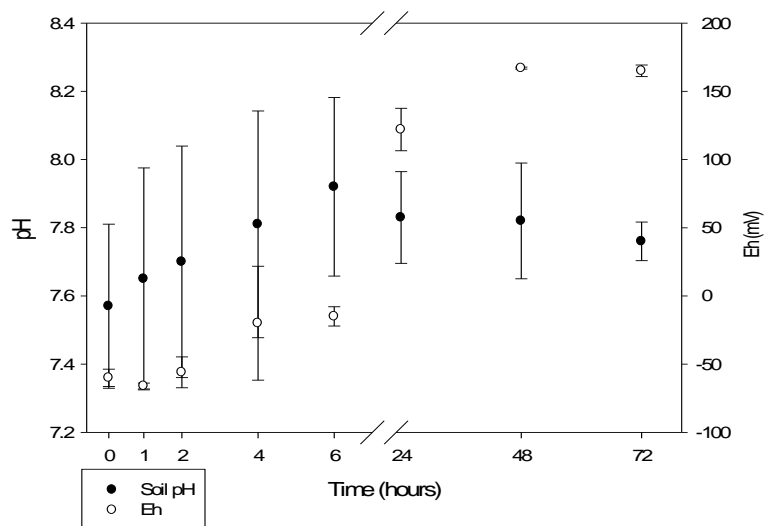


Figure 2.8: Soil slurry pH and Eh under anoxic conditions after NaNO_3 addition (error is standard deviation of mean data point).

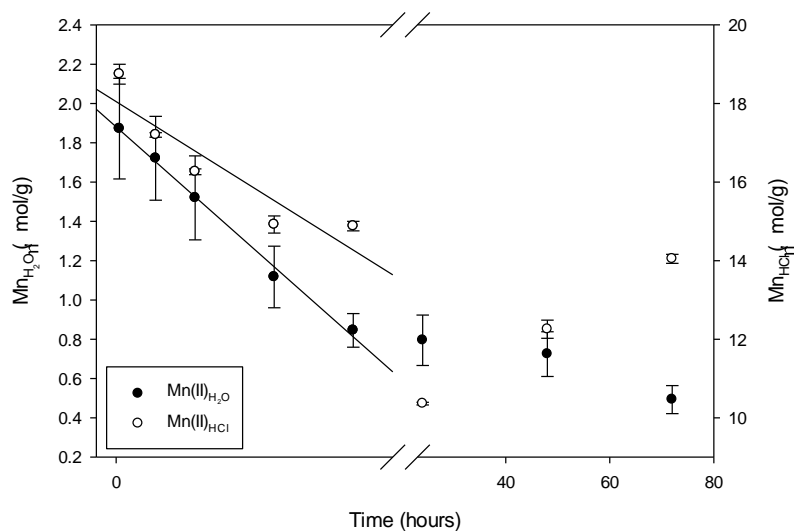


Figure 2.9: Water and acid extractable Mn(II) immediately following NaNO_3 addition. (Water extractable $\text{Mn(II)} = -0.178x + 1.88$; $R^2 = 0.994$ and acid extractable $\text{Mn(II)} = -0.730x + 18.05$; $R^2 = 0.847$; error is standard deviation of mean data point)

The concentration of Fe(II), both water and acid extractable, also dropped during the first 6 hours post nitrate addition (Figure 2.10). The rates of Fe(II) disappearance in the water and HCl extractable fractions were $0.055 \mu\text{mol g}^{-1} \text{ hour}^{-1}$ and $1.63 \mu\text{mol g}^{-1} \text{ hour}^{-1}$,

respectively. Similar to Mn(II) oxidation, the removal of Fe(II) was not due to significant increases in E_h during the first 6 h, which agrees with previous work on the Sadler soil (Matocha and Coyne, 2007).

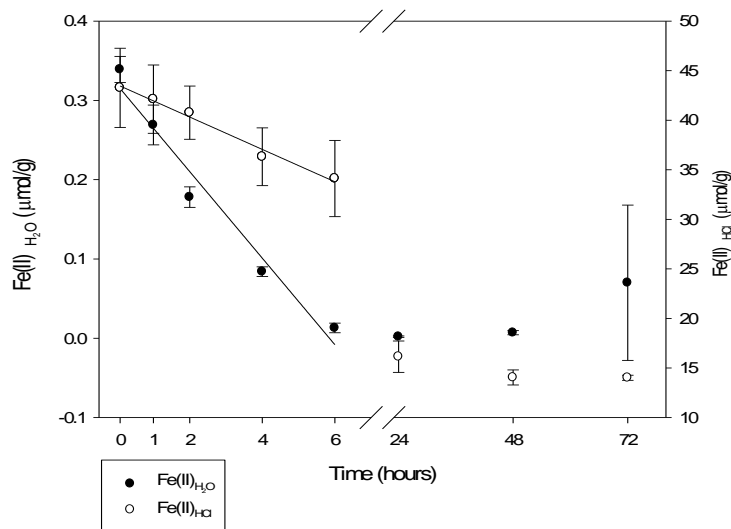
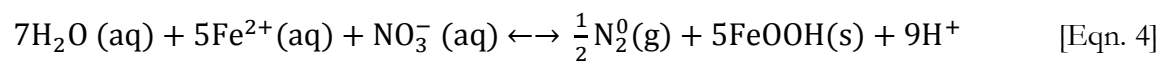


Figure 2.10: Concentration of water and acid extractable Fe(II) immediately following NaNO_3 addition (Water extractable $\text{Fe(II)} = -0.055x + 0.319$; $R^2 = 0.966$ and acid extractable $\text{Fe(II)} = -1.63x + 43.95$; $R^2 = 0.985$; error is standard deviation of mean data point).

One of the objectives of this research was to estimate the contribution of Fe(II) oxidation to nitrate reduction. This was accomplished using the rates of consumption of Fe(II) and NO_3^- , determined as the slopes of the linear least squares fit of water-soluble Fe(II) and NO_3^- , from Figures 2.6 and 2.10. In addition, we assumed that the reaction stoichiometry in Equation 4 is as follows:



The following steps show how the contribution was calculated:

Step 1: Establish the electron transfer from Equation 4

$$5 \text{ e}^- \text{ transfer}$$

Step 2: Compare the initial slopes of Fe(II) oxidation and NO_3^- reduction

$$\frac{\Delta\text{Fe}^{2+}}{\Delta\text{NO}_3^-} = \frac{-0.055}{-0.0276}$$

Step 3: Divide by the number of electrons transferred

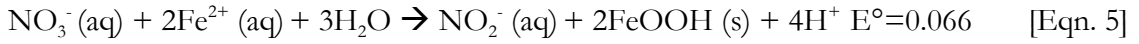
$$\frac{\frac{\Delta Fe^{2+}}{\# \text{ electrons}}}{\Delta NO_3^-} = \frac{\frac{-0.055}{5}}{-0.0276} = .398$$

Step 4: Multiply by 100 to establish a percentage

$$\frac{\frac{\Delta Fe^{2+}}{\# \text{ electrons}}}{\Delta NO_3^-} = \frac{\frac{-0.055}{5}}{-0.0276} = .398(100) = 39.8\%$$

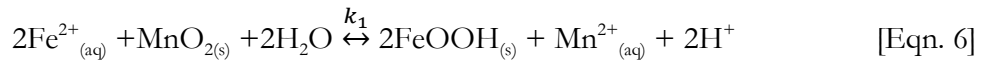
From these steps, it was determined that the contribution of Fe(II) oxidation to the reduction of NO_3^- was 39.8%.

Half reactions describing nitrate reduction to nitrite were coupled to iron(II) oxidation, assuming goethite as the reaction product, results in the following equation [Eqn. 5]



With the pH, nitrate, nitrite, and Fe^{2+} concentrations from this experiment, the Nernst equation was used to establish the E_{cell} of the reaction. Then, the Gibbs free energy was calculated to be $\Delta G = -168.3 \text{ kJ/mol}$, indicating that the oxidation of Fe(II) was highly favorable under these control conditions.

The greater Mn(II) oxidation rates, compared with Fe(II) oxidation (compare Figures 2.9 and 2.10) during nitrate reduction, suggests that the secondary reaction involving dissolved Fe(II) and freshly precipitated Mn(III, IV) oxides might be operative. This might account for a portion of the dissolved Fe(II) removed from solution, that is currently attributed to NO_3^- alone. This overall reaction has been described by the following equation (Postma, 1985):



where $MnO_{2(s)}$ is a representative Mn oxide mineral assumed to be freshly precipitating in our slurries, and $FeOOH_{(s)}$ is goethite. This reaction has been shown to follow a second order kinetic rate expression (Postma, 1985; Edwards, 2007):

$$\frac{[Fe^{2+}_{(aq)}]}{dt} = -2k_1 [Fe^{2+}_{(aq)}]_o [MnO_{2(s)}]_o \quad [\text{Eqn. 7}]$$

where $\frac{[Fe^{2+}_{(aq)}]}{dt}$ is the rate of disappearance of dissolved Fe(II), $[Fe^{2+}_{(aq)}]_o$ is the initial Fe(II) concentration, $[MnO_{2(s)}]_o$ is the Mn oxide concentration, k_1 is the second order rate coefficient ($L \text{ mol}^{-1} \text{ h}^{-1}$), and the factor 2 reflects the stoichiometry in Eqn. 6. Equation 7 can be integrated to solve for dissolved Fe(II) as a function of time:

$$Fe_{(aq)}^{2+} t = \frac{Fe_{(aq) o}^{2+}}{-1 + 2e^{-Fe_{(aq) o}^{2+} k_1 t}} \quad [\text{Eqn. 8}]$$

where $Fe_{(aq) o}^{2+}$ was taken as the value obtained at the end of the preincubation (see Figure 2.3) and k_1 values were utilized from studies performed by Postma (1985) and Edwards (2007), respectively. A plot of $Fe_{(aq)}^{2+} t$ over time for a k_1 value of 1140 L/mol h (from Edwards, 2007) is shown in Figure 2.11A, along with observed $Fe_{(aq)}^{2+}$ values from the Sadler control experiments. It is clear that this secondary reaction is relevant in the time scales of our experiments. Using the rates determined in Figure 2.11B, with corrected values of Fe^{2+} , the contribution of Fe^{2+} oxidation to nitrate reduction was reduced to 19%. We regard this correction as only an estimate, but it serves to illustrate the importance of the secondary reaction described in Eqn. 6. The value of k_1 from Postma's (1985) study was 2283 L/mol h, which would further lower the contribution of Fe^{2+} oxidation to nitrate reduction (data not shown).

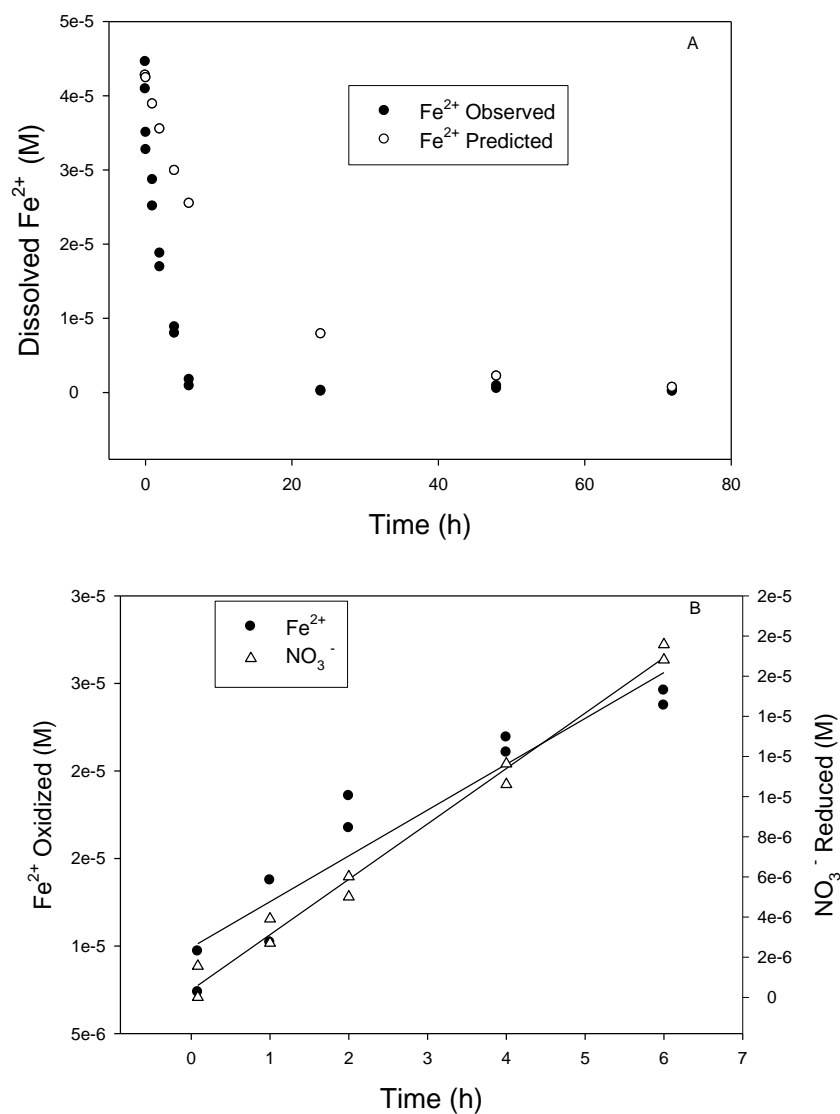


Figure 2.11: A. Water-soluble Fe^{2+} values predicted using [Eqn. 8] and a k_1 value of 1140 L/mol h as compared with observed values from the Sadler soil. B. Corrected concentration values of oxidized Fe^{2+} as compared with concentrations of reduced nitrate (Water extractable $\text{Fe}^{2+} = 2.618\text{E}^{-06}x + 9.912\text{E}^{-06}$, $R^2 = 0.89$; NO_3^- Reduced = $2.759\text{E}^{-06}x + 3.651\text{E}^{-07}$, $R^2 = 0.98$).

Following the concentrations of acetate and carbon dioxide during the reaction allowed us to explore the role of carbon during nitrate-dependent Fe(II) oxidation. Despite the initial excess of acetate over nitrate, acetate concentration remained constant after nitrate addition during the first six hours (Figure 2.12). This constant concentration, over the first six hours post nitrate addition, suggests that acetate was not used as a primary electron

donor or carbon source early in the reaction. This is surprising, given that acetate is reported to serve as a good substrate for nitrate reduction (Van Rijn et al., 1996; Chidthaisong and Conrad, 2000; André et al., 2011). Other dissolved organic carbon phases that were not measured may have been utilized, as opposed to acetate, during the first six hours of reaction.

The drop in acetate concentrations commensurate with complete depletion of nitrate after 6 h and beyond could be due to participation of heterotrophic denitrifying bacteria. Heterotrophic denitrifiers can experience a lag phase, requiring time to produce enzymes necessary for denitrification (Smith and Tiedje, 1979). It is also possible that mixotrophic Fe(II) oxidizing bacteria were consuming acetate concurrent with nitrate reduction. Muehe et al. (2009) reported that maximum growth of a mixotrophic Fe(II) oxidizing nitrate reducing microorganism occurred with an Fe(II) plus acetate treatment, when compared with acetate alone.

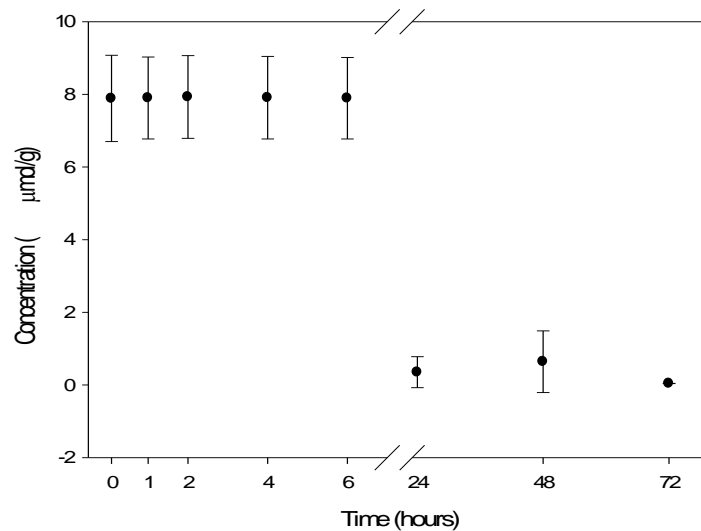


Figure 2.12: Concentration of acetate after the addition of NaNO_3 (error is standard deviation of mean data point).

Carbon dioxide was also followed after the addition of nitrate. The cumulative CO_2 data is shown as total dissolved inorganic carbon (DIC) in Figure 2.13, below.

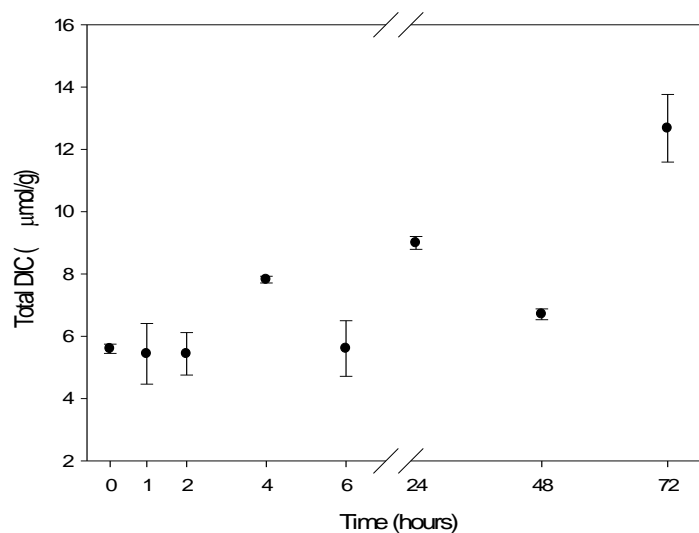


Figure 2.13: Cumulative total dissolved inorganic carbon (DIC) after the addition of NaNO_3 under anoxic conditions (error is standard deviation of mean data point).

Carbon dioxide evolution for the first two hours after nitrate addition was minimal.

However, after 24 hours, greater evolution of gas was found. The increase in CO_2 production corresponds with the disappearance of acetate. It has been shown that acetate can be oxidized to CO_2 , providing evidence that acetate may have been used as an electron donor at later times in the reaction (Chidthaisong and Conrad, 2000).

Manganese(II) Oxidation Contribution to Nitrate Reduction

Preincubation

Because soil was treated identically during the preincubation stage of ferrozine-treated experiment, the preincubation trends were very similar to the preincubation trends found in the control experiment. These trends are shown in Figure 2.14 (a-e), below.

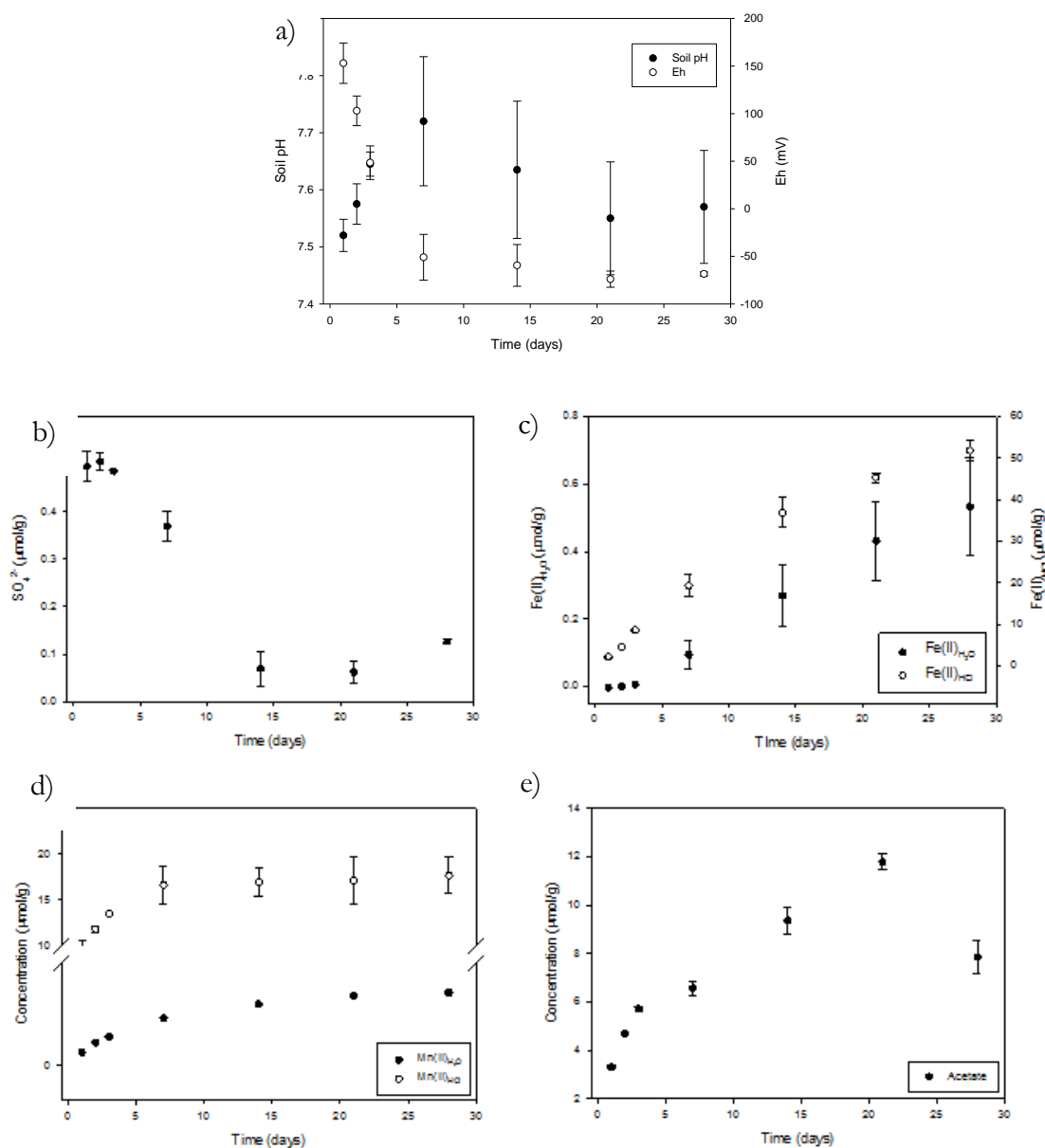


Figure 2.14: Ferrozine amended soil pH and Eh (a); sulfate concentration (b); water and HCl extractable Fe(II) concentrations (c); water and HCl extractable Mn(II) concentrations (d); and acetate concentration (e) during anaerobic preincubation (error is standard deviation of mean data point).

Post Nitrate Addition

Before the addition of 0.1mM NaNO_3 to the soil slurry, 1mM Ferrozine was added to the slurry to ensure that the native Fe(II) was complexed, essentially eliminating Fe(II) from the reaction. Once nitrate was added, there was an immediate drop in nitrate

concentration over the first 6 hours (Figure 2.15). Nitrate disappearance occurred at a rate of $0.057\mu\text{mol g}^{-1} \text{ hour}^{-1}$ over the first 6 hours and then went to nearly the detection limit after 24 hours. This rate of nitrate reduction was two-fold faster than in the control experiment ($0.028\mu\text{mol g}^{-1} \text{ hour}^{-1}$). Nitrite concentration was negligible, with no appearance after the addition of nitrate (Figure 2.15).

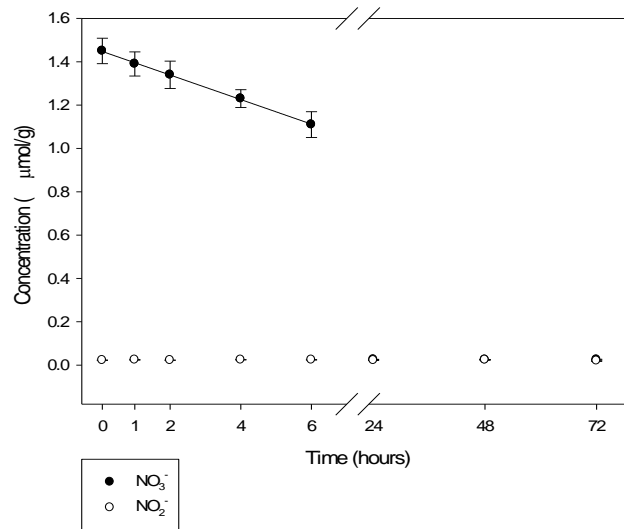


Figure 2.15: Ferrozine amended soil nitrate and nitrite concentrations after NaNO_3 addition ($y = -0.057x + 1.45$; $R^2 = 0.999$; error is standard deviation of mean data point).

There was an appearance of nitrous oxide, increasing up to almost $3\mu\text{mol g}^{-1}$, during the first 6 hours post nitrate addition, after which this gas dropped back down to nearly the detection limit (Figure 2.16).

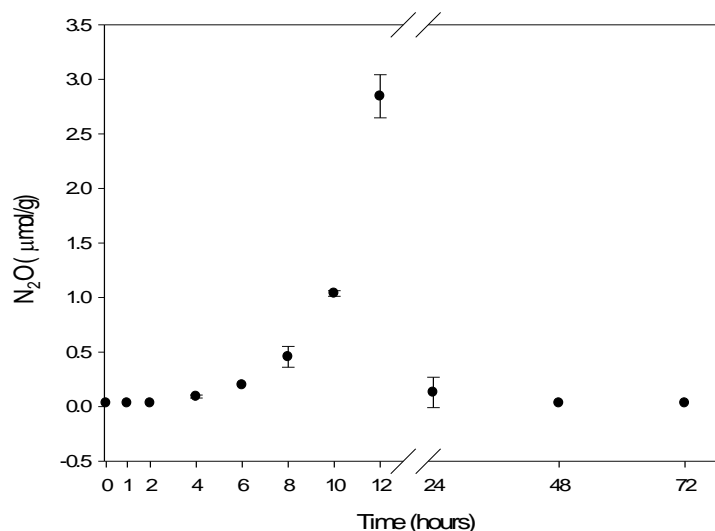


Figure 2.16: Nitrous oxide concentration after nitrate addition to ferrozine-amended soil slurries (error is standard deviation of mean data point).

The drop in nitrate concentration with the transient appearance of nitrous oxide indicates that nitrate reduction is proceeding all the way to N₂. As in the control, no ammonium was detected (data not shown).

While the nitrate concentration was rapidly decreasing, there was a decrease in the concentration of water extractable Mn(II) at a rate of $0.036 \mu\text{mol g}^{-1} \text{ hour}^{-1}$, whereas HCl extractable Mn(II) remained almost constant (Figure 2.17). The rate of water-soluble Mn(II) oxidation in the ferrozine-amended soil was much less than the control (see Figure 2.9, $0.178 \mu\text{mol g}^{-1} \text{ hour}^{-1}$). Apparently, complexing Fe(II) and preventing precipitation to poorly crystalline Fe(III) minerals during nitrate reduction as that described in Eqn. 4 might have removed a sink for Mn(II). Mn(II) is known to adsorb to Fe(III) oxide minerals (Junta and Hochella, 1994) and this might explain the greater rate of Mn(II) removal in the control, where Fe(II) was actively oxidizing.

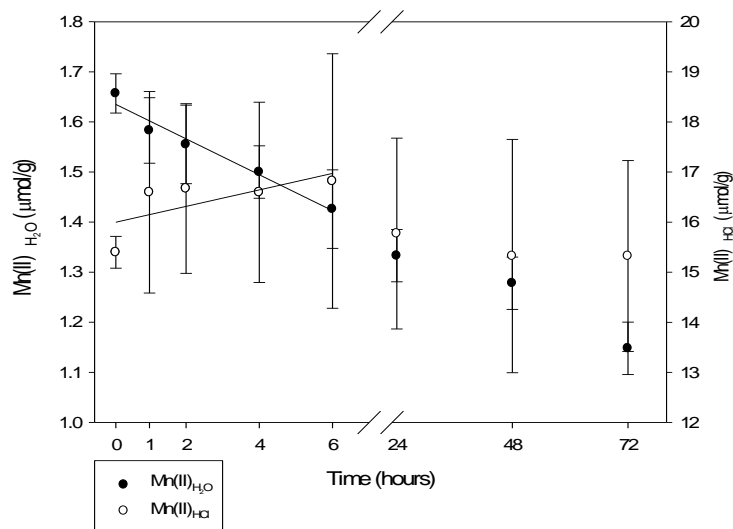
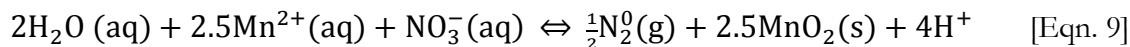


Figure 2.17: Ferrozine amended soil water and HCl extractable Mn(II) concentrations post nitrate addition (Water extractable Mn(II) = $-0.036x + 1.638$; $R^2 = 0.966$ and acid extractable Mn(II) = $0.1647x + 15.98$; $R^2 = 0.464$; error is standard deviation of mean data point)

Using the ferrozine-amended Mn(II) oxidation rates, the nitrate reduction rates, and assuming that Equation 9, below, holds, the contribution of Mn(II) oxidation to nitrate reduction was 25.2%.



Furthermore, from the half-cell reactions of nitrate reduction to nitrite and manganese oxidation to manganese(II) oxide, Equation 10 below was used to establish a ΔG for our reaction conditions.



Under our stirred batch conditions, the $\Delta G = -15.75$ kJ/mol. This indicates that this reaction was favorable under our anaerobic stirred batch conditions. Our ΔG is very similar to that obtained by Luther et al. (1997), in which the same reaction was postulated, over a range of pH values, to have a $\Delta G_f = -14.20$ kJ mole⁻¹. The Mn(II) reaction had a less spontaneous ΔG than the Fe(II) reaction, $\Delta G = -168.3$ kJ/mol, shown previously (Equation 5).

To our knowledge, this is the first time nitrate-dependent Mn(II) oxidation has been confirmed in soil. Thermodynamic evidence suggests some abiotic driving force, but another proposed explanation is metal-reducing bacteria of the genus *Shewanella* utilizing

Mn(II) as an electron donor and coupling this to NO_3^- reduction. Previous studies have reported that NO_3^- immediately inhibited Mn(IV) reduction to Mn(II) by 50% when added to a pure culture of *Shewanella* (Myers and Nealson, 1988). One might infer that this was due to Mn(II) oxidation coupled to NO_3^- reduction and this has been proposed elsewhere (Luther et al., 1997). This process was speculated to account for patterns in Mn(II) oxidation in two other studies (Tebo, 1991; Oguz et al., 2001). Field evidence points to the possibility of *Shewanella* sp. playing an active role in reoxidizing end products of their respiration (namely, Mn(II)) (Staudigel et al., 2006; Bräuer et al., 2011). Bräuer et al. (2011) further speculate that the ability of *Shewanella* to oxidize Mn(II) might be a way of storing electron acceptors (oxidized Mn(IV) oxides) to cope with fluctuating redox conditions. It is possible that *Shewanella* species are present, as they have been identified in diverse soil environments with fluctuating redox status (DiChristina et al., 2005; DeAngelis et al., 2010). The next logical step would be to characterize the microorganisms present in the Sadler soil, given that these patterns in Mn(II) and nitrate behavior are present. Studies such as these warrant further investigation.

The concentration of water extractable Fe(II) remained fairly constant at almost $2.5\mu\text{mol g}^{-1}$ while the acid extractable Fe(II) decreased in concentration from $0.75\mu\text{mol g}^{-1}$ to approximately $0.55\mu\text{mol g}^{-1}$ over the first 6 hours after nitrate addition (Figure 2.18). These constant concentrations of Fe(II) were expected with the addition of ferrozine, which was supposed to block the reaction of iron(II) with other species after the addition of nitrate. The greater concentration of water extractable Fe(II) than acid extractable Fe(II) differs from the trends seen in the control experiments. Higher levels of water extractable Fe(II) may be explained by the addition of ferrozine. Ferrozine is a strong binding agent for iron. Consequently, when ferrozine was added to the soil slurry, it may have pulled iron from sorbed sites to form a complex. When an excess of ferrozine is added to solution, complete Fe(II) complexation has been observed (Sarradin et al., 2005). As a result, the concentration of water extractable Fe(II) would be greater than that of the acid extractable Fe(II) because the sorbed Fe(II) that is usually extracted by HCl would mostly be complexed by the ferrozine.

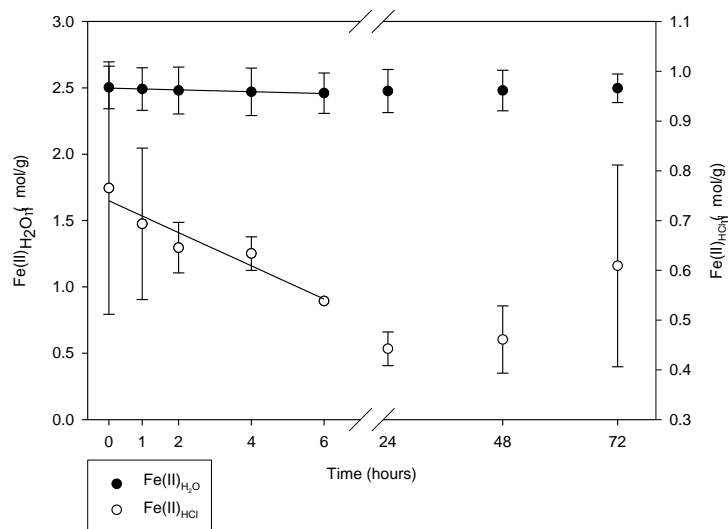


Figure 2.18: Ferrozine amended soil water and HCl extractable Fe(II) concentrations post nitrate addition. (Water extractable Fe(II) = $-0.0069x + 2.499$; $R^2 = 0.950$ and acid extractable Fe(II) = $-0.0334x + 0.743$; $R^2 = 0.913$; error is standard deviation of mean data point)

As shown previously, the pH of the soil slurry on the 28th day of preincubation was 7.52. However, five minutes after the addition of nitrate, the pH was 7.3, shown in Figure 2.19. This sharp initial drop in pH may have been due to the addition of ferrozine before the addition of nitrate. Because ferrozine has a $pK_a=3.13$ (Thompson and Mottola, 1984), this may have induced a sudden drop in pH. But, after the addition of nitrate, the pH increased consistently over the remaining time of the experiment, to about pH 7.8 (Figure 2.19). This is consistent with Equation 3, in which a reduction of nitrate would promote an increase in pH. The Eh also had an immediate change in the first 5 minutes post nitrate addition. The Eh jumped from -68mV to almost 0mV where it remained constant until 24 hours after nitrate addition (Figure 2.19). After 48 and 72 hours, the Eh dropped slightly, to approximately -30mV. This post nitrate addition Eh trend varies from that of the control experiment. In the control experiment, Eh increased gradually after nitrate addition, whereas in this ferrozine-amended experiment, Eh remained constant and even declined slightly. With ferrozine binding Fe(II) to eliminate Fe(II) reactions, a less dramatic change in redox potential occurred, indicating that iron oxidation may be a significant factor in increasing redox potential.

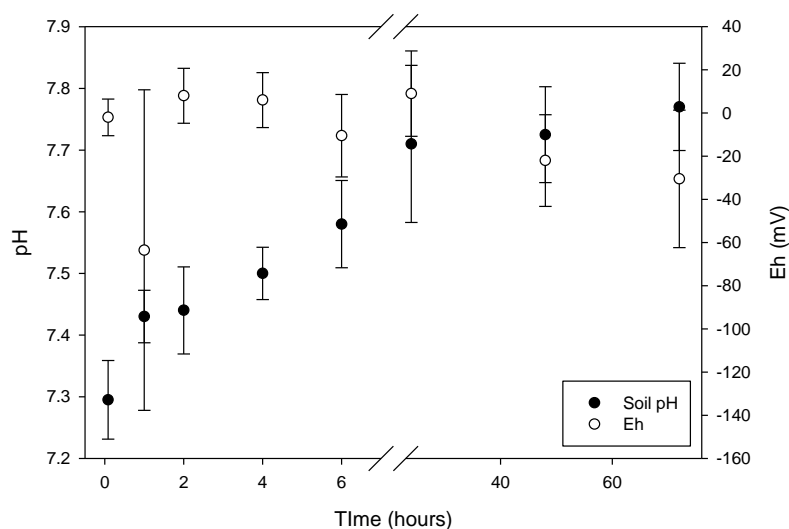


Figure 2.19: Ferrozine amended soil slurry pH and Eh after nitrate addition (error is standard deviation of mean data point).

The concentration of acetate on day 28 of preincubation was almost $8\mu\text{mol g}^{-1}$ and after the addition of nitrate, the acetate concentration dropped slightly, to about $7\mu\text{mol g}^{-1}$, remaining constant until 24 hours post nitrate addition (Figure 2.20). After 48 hours, the concentration of acetate increased slightly, to a little over $9\mu\text{mol g}^{-1}$ (Figure 2.20). As in the control experiment, there was little utilization of acetate as an electron donor in the first 24 hours after nitrate addition. This may indicate preferential use of manganese as the electron donor.

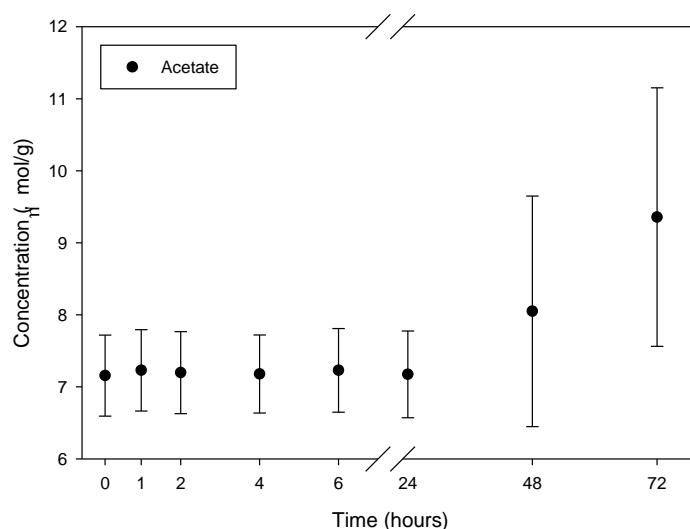


Figure 2.20: Acetate concentration post nitrate addition to ferrozine-amended soil (error is standard deviation of mean data point).

Furthermore, the increase in acetate concentration after 24 hours post nitrate addition may be due to fermentation, as described in Conrad and Klose (2011).

Though acetate was constant over the first 24 hours post nitrate addition, there was an increasing concentration of total DIC, from $1.5\mu\text{mol g}^{-1}$, to almost $2.6\mu\text{mol g}^{-1}$, followed by a small decrease over the next two days (Figure 2.21). Because acetate was not utilized as an electron donor post nitrate addition, this rise in DIC may be due to consumption of a low molecular weight organic acids other than acetate by heterotrophic denitrifiers. If so, this suggests that there may be some competition between Fe(II) oxidizers and heterotrophic microorganisms for available nitrate, because the rate of nitrate reduction was faster and DIC consistently increased where Fe(II) is prevented from reacting (in ferrozine amended slurries), as compared with the control, where minimal changes in DIC occurred.

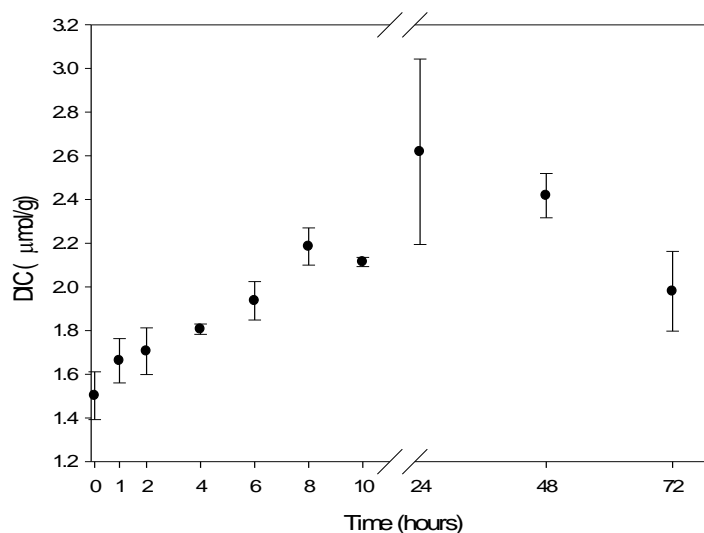


Figure 2.21: Ferrozine amended soil total cumulative dissolved inorganic carbon (DIC) post nitrate addition (error is standard deviation of mean data point).

CONCLUSIONS

By allowing all of the native soil Fe(III) to reduce to Fe(II), followed by nitrate addition, we were able to quantify the contribution of Fe(II) oxidation to nitrate reduction in the Sadler silt loam. Iron oxidation via nitrate addition under anoxic conditions was rapid and thermodynamically favorable over the first six hours after nitrate addition in the control experiments. Oxidation of Fe(II) contributed 40% to the reduction of nitrate. Correcting the water-soluble Fe(II) values for the abiotic secondary reaction involving MnO_2 lowered the value to 19%. Where these contributions were calculated during the first 6 h, there was no evidence that acetate was utilized as either an electron donor or a carbon source. It is possible that a combination of biological processes and abiotic processes are involved in this coupled process. Lithotrophic and mixotrophic microorganisms have been identified that can couple Fe(II) oxidation to nitrate reduction (Roden, 2012). Assuming that reactive Fe(II) minerals such as green rust are absent in our systems (the only abiotic pathway to reduce nitrate at measureable rates), another possibility is a coupled biological-abiotic process. This might involve nitrate reduction to nitrite by lithotrophic microorganisms followed by abiotic Fe(II) oxidation coupled to nitrite reduction (Roden, 2012; Matocha et al., 2012). Nitrite is much more reactive towards Fe(II) in abiotic systems than is nitrate.

At longer reaction times in the Sadler control slurries (>6 h), acetate was removed from solution and there was a general pattern of increasing carbon dioxide production, based on measured increases in dissolved inorganic carbon. It is possible that heterotrophic denitrifiers were active in nitrate reduction, as well as mixotrophic microorganisms, because Fe(II) continued to decline.

For the first time, it was documented that Mn(II) oxidation was coupled to nitrate reduction. This was assessed by the addition of ferrozine after 28 days of preincubation. Ferrozine successfully bound native Fe(II) to eliminate it from being oxidized by Mn(III, IV)-oxides. This allowed the calculation of the contribution of Mn(II) oxidation to nitrate reduction to be more accurate. Results indicated that the oxidation of Mn(II) contributed 25.2% to the reduction of nitrate in these stirred-batch reactions.

The sum of the contributions of Fe(II) and Mn(II) oxidation to nitrate reduction was 44%. Although initial soil organic carbon in the Sadler soil was in a three-fold excess over total Fe ($1083.3 \mu\text{mol g}^{-1}/333.5 \mu\text{mol g}^{-1}$) and a 68-fold excess over total Mn ($1083.3 \mu\text{mol g}^{-1}/15.9 \mu\text{mol g}^{-1}$), these inorganic elements account for nearly one-half of the nitrate reduced. The remainder might be attributed to heterotrophic denitrification. These results demonstrate that native soil Fe(II) and Mn(II) can serve as electron donors for nitrate reduction and merit further consideration in denitrification studies, where soil organic carbon has long been held as the primary electron donor in this process.

CHAPTER 3: IMPACT OF HYDROGEN GAS AND PLANT RESIDUE AMENDMENTS ON NITRATE DEPENDENT IRON(II) AND MANGANESE(II) OXIDATION

Results from Chapter 2 were obtained with the Sadler's native terminal electron acceptors (TEAs) and donors. Nitrate was added under anoxic conditions as a TEA to simulate a fertilizer addition and it was found that nitrate was reduced concomitantly with Fe(II) and Mn(II) oxidation. Many studies that have explored how various amendments that supply electron donors and electron acceptors alter anaerobic processes (Lovley and Goodwin, 1988; Chidthaisong and Conrad, 2000; Liptzin and Silver, 2009; Salas et al., 2009; Conrad and Klose, 2011). However, insight into specific changes for both manganese and iron pools, with the addition of certain amendments, are still not well known.

While nitrate acts as the main electron acceptor in nitrate-dependent Fe(II) oxidation, effects of the presence of another electron donor in conjunction with nitrate are worthy of research to explore potential changes in Fe(II) and Mn(II) oxidation contributions to nitrate reduction. The results of Iannotti et al. (1973) confirmed that H₂-utilizing organisms could cause electrons to be shifted away from production of a typical fermentation product in favor of the more oxidized product. These findings, in combination with others, resulted in the establishment of what they deemed "interspecies electron transfer", mediated by H₂ gas, which is proposed to affect which products are formed under anaerobic conditions (Iannotti et al., 1973). Under anaerobic conditions, bacterially mediated organic matter fermentation results in CO₂ and H₂ products (Dolfing, 1988). Once present, H₂ can reduce inorganic electron acceptors via bacteria (Zinder, 1993). The H₂ also acts as a couple for oxidative and reductive processes in general (Hoehler et al., 1998). In addition, varying H₂ concentrations under laboratory conditions showed significant effects on the flow of electrons during organic matter decomposition (Hoehler et al., 1998). Hydrogen production and consumption in sediments where Fe(III) reduction is the terminal electron accepting process has also been shown (Lovley and Phillips, 1987). Rates of sediment Fe(III) reduction under anaerobic atmospheres with hydrogen were faster than when hydrogen was not present in the reaction atmosphere (Lovley and Goodwin, 1988). Furthermore, there has also been evidence that hydrogen oxidation can be coupled to nitrate

and Mn(IV) reduction in sediments (Lovley and Goodwin, 1988), due to hydrogen disappearance when Mn(IV) and nitrate were added to sediment. However, these potential couplings have not been well explored. In a heterogeneous mixture such as a soil slurry, where organic matter is likely present, H_2 would act as an ideal electron donor because H_2 can act as an effective substrate in the reduction of inorganic electron acceptors.

No-tillage (NT) is the agricultural practice of leaving residue from the previous crop on the soil surface to prevent erosion, increase yields, and promote water capture, among other benefits (Havlin et al., 2005). No-tillage is widely used in Kentucky in the production of corn, soybeans, and small grains. The practice of leaving plant residue on the soil surface has become increasingly adopted over the past 50 years, especially because of its influence on nitrogen in the soil. Therefore, effects of plant residue addition on nitrate-dependent Fe(II) and Mn(II) oxidation were worth exploring.

Denitrification has been historically associated with soil carbon. Organic carbon not only acts as an energy source for microbial activity, but also acts as an electron donor (Lescure et al., 1992). Greater reducing conditions have been shown to be induced when citrate and malate are added to soil (Lescure et al., 1992). Glucose and acetate also promote reducing conditions, but are not as reducing as citrate and malate (Lescure et al., 1992). Nitrate reduction in these experiments was attributed more to the carbon than the lowering of the Eh (Lescure et al., 1992). Research by Paul et al. (1989) determined that adding the carbon sources acetate, propionate, and butyrate resulted in a positive correlation between denitrification with respect to the available electrons per mole of carbon. Furthermore, adding glucose and sucrose lacked correlation with denitrification capacity, possibly because of competition between fermentative microbes and denitrifiers (Paul et al., 1989). Nonetheless, mineralizable and water-soluble organic carbon are strongly associated with increasing denitrification capacity (Burford and Bremner, 1975). Increased denitrification capacity, in this case, was determined by the amount of $(N_2O + N_2)$ -N evolved (Burford and Bremner, 1975). Furthermore, readily decomposable organic matter is a reflection of the amount of mineralizable carbon present. So, it could be proposed that plant residue, acting as the readily decomposable organic matter, would increase the denitrification capacity in stirred batch experiments, in turn effecting the various contributions and rates of nitrate-dependent Fe(II) and/or Mn(II) oxidation.

Furthermore, differences between the types of residue added may present varying effects on nitrate-dependent Fe(II) and/or Mn(II) oxidation. Studies by Paul and Beauchamp (1989) compared denitrification rates in soils amended with various plant residues including alfalfa, red clover, corn stover, and wheat straw. Additionally, they manipulated C:N ratios of the treatments (Paul and Beauchamp, 1989). Denitrification in Paul and Beauchamp's (1989) experiments was measured as N₂O accumulation. Nitrous oxide accumulation was fastest among the high-N alfalfa and red clover amended soils (Paul and Beauchamp, 1989). High-N-treated corn stover and wheat straw amended soils exhibited slower accumulation of N₂O and had greater amounts of nitrate remaining in them after 15 days of incubation (Paul and Beauchamp, 1989). While these experiments compared denitrification among plant residues, they did not provide a comparison of denitrification rates of plant residue amended soil with denitrification rates of an un-amended soil.

It is possible that adding plant residues with readily available organic carbon stimulates production of Fe(II) and Mn(II) and might actually lead to a greater contribution of these elements to nitrate reduction. In the past, these additions catalyzed nitrate reduction and this has been attributed to greater activity of heterotrophic denitrifiers, rather than involvement of iron or manganese. Therefore, the objectives of this study are to evaluate the impact of H₂ and plant residue addition on nitrate-dependent Fe(II) and/or Mn(II) oxidation in the Sadler soil. It is hypothesized that adding H₂ and plant residues will have opposite effects on nitrate-dependent Fe(II) and Mn(II) oxidation; H₂ will compete with Fe(II) and Mn(II) as electron donors and lower their contribution whereas plant residue will increase their contribution to nitrate reduction.

MATERIALS AND METHODS

Hydrogen Amendment

Protocol for this section of experimentation is the same as the methods described in the "Fe(II) Oxidation Contribution to NO₃⁻ Reduction" section of materials and methods in Chapter 2. However, to explore the effects of an additional electron donor, the anaerobic atmosphere was a mixture of argon (95%) and hydrogen (5%) gases.

Plant Residue Amendment

A single cultivar of red winter wheat (Pioneer 25R32) that had been amended with nitrogen fertilizer at rates of 0 lb N/acre (acting as the high C:N ratio) and 150 lb N/acre (acting as the low C:N ratio) was harvested from Dr. John Grove's plots at the University of Kentucky's Spindletop Research Farm (Lexington, KY). The winter wheat was harvested at late boot growth stage, dried at 60°C, ground, and stored in a dessicator. The C:N ratio of the 0 lb N/acre residue was 16.71 while the C:N ratio of the 150 lb N/acre residue was 16.15.

Stirred-batch reactions were set up as previously described: 14g of Sadler soil in a 160mL glass serum bottle and 10g of Sadler soil in a 160mL glass serum bottle. Wheat residue was added to each bottle at a rate of 2mg dried residue g⁻¹ soil before the addition of water in the anaerobic glove box. The amount of residue added was derived from anaerobic stirred batch reactions conducted by Conrad and Klose (2011). After adding of the wheat residue, stirred-batch experiments were conducted and analyzed as described in the "Fe(II) Oxidation Contribution to NO₃⁻ Reduction" materials and methods section (Chapter 2). Treatments were duplicated for each residue from each fertilizer rate (high C:N ratio and low C:N ratio) under an Argon gas atmosphere.

RESULTS AND DISCUSSION

Hydrogen Amendment

Preincubation

The pH of the soil slurry rose from pH 7.6 to pH 7.8 over the first 7 days, then dropped to approximately pH 7.65 (Figure 3.1) over the course of the 28-day preincubation. The Eh decreased from approximately 50mV to a reducing redox potential of about -125mV (Figure 3.1). Trends in pH in the control (Argon atmosphere only, with no amendments) experiment resulted in a pH rise to pH 7.8, whereas in this case, with the added hydrogen in the atmosphere, the final pH after 28 days was 7.65. A lower final pH would be expected with the addition of hydrogen to the anaerobic atmosphere, because added hydrogen would contribute to an increase in hydrogen ion concentration, resulting in lower pH. Additionally, the Eh dropped to a much more negative potential in the hydrogen-amended experiment than in the control experiment where the 28-day redox potential only reached approximately

50mV. The redox potential could be more negative in this case due to activity of Fe(III) and Mn(III, IV) reducing bacteria utilizing hydrogen (Lovley and Goodwin, 1988).

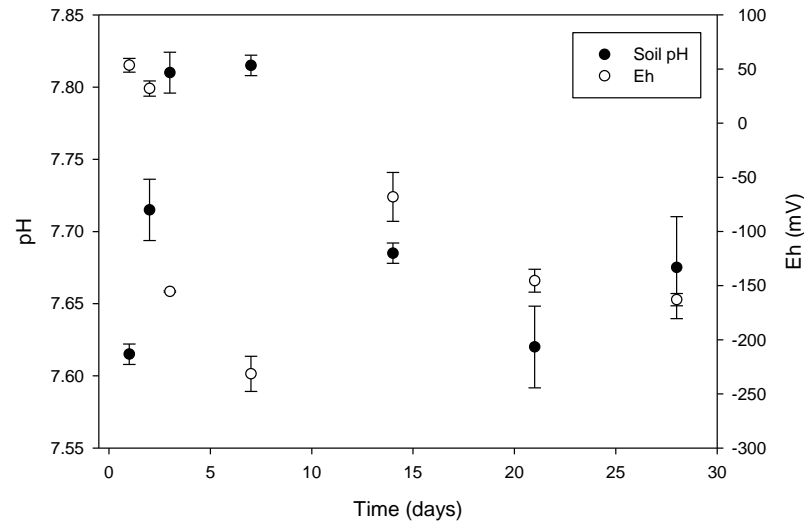


Figure 3.1: Soil pH and Eh under hydrogen amended anoxic conditions during preincubation (error is standard deviation of mean data point).

Manganese(II) concentrations, both water and acid extractable, increased rapidly over the first 14 days of preincubation (Figure 3.2). Manganese(II) concentrations peaked at 14 days, whereas peak Mn(II) production was reached at 21 days in the control experiment (Figure 2.2). This indicates that native manganese was reduced to manganese(II) fairly early in the incubation, likely due to the more negative redox potential achieved during this preincubation. Furthermore, because all other species had reduced, Mn(III, IV) was the next species to reduce, which occurred by day fourteen. Adding hydrogen has been shown to couple with Mn(IV) reduction in sediments, so a greater rate of reduction would be expected (Lovley and Goodwin, 1988). This reduction of Mn(IV) has been attributed to the activity of microorganisms (Lovley and Goodwin, 1988). Therefore, the drop in Mn(II) concentration after 14 days, may have been due to a lack of hydrogen-consuming, Mn(III, IV)-reducing microbes remaining active in the native soil.

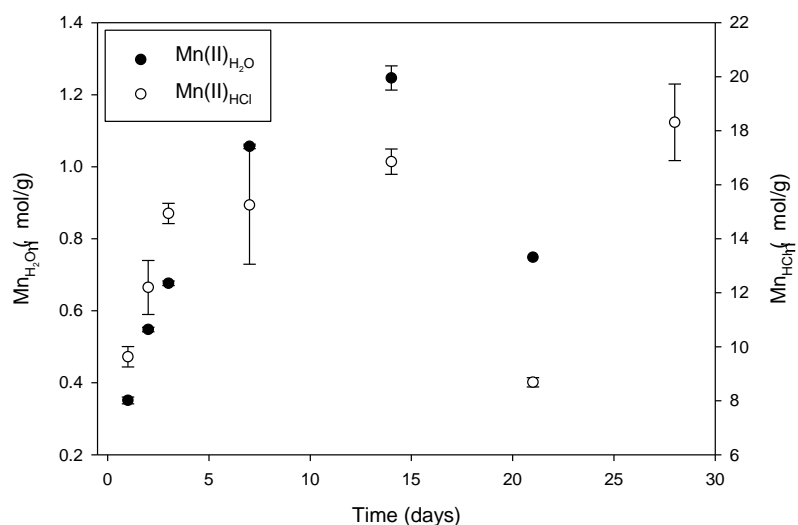


Figure 3.2: Water and HCl extractable Mn(II) concentrations under hydrogen amended anoxic conditions during preincubation (error is standard deviation of mean data point).

During preincubation, water and acid extractable Fe(II) concentrations increased. As in the previous experiment (Figure 2.3), the acid extractable Fe(II) concentration was 100x greater than the water extractable Fe(II) concentration. Iron(III) reduction was completed by day 28 of preincubation. Because there was rapid reduction of manganese during the first part of preincubation, this may have induced a lag in the reduction of native iron(III). The lag in total Fe(III) reduction is to be expected because Fe(III) follows Mn(III, IV) in the redox sequence. The initial and final values of Fe(II) were very similar to those of the control, increasing from nearly $0\mu\text{mol g}^{-1}$ to $0.4\mu\text{mol g}^{-1}$ (Figure 3.3). However, the rate of reduction, to allow all native Fe(III) to reduce to Fe(II), was slower in the hydrogen-amended experiments than in the control. This slower rate of reduction was not expected as results, previously mentioned (Lovley and Goodwin, 1988; Achtnich et al., 1995) demonstrated that adding hydrogen would result in a faster rate of Fe(III) reduction. A slower reduction rate in this experiment may have been due to the presence of other terminal electron acceptors also utilizing electrons from the added hydrogen.

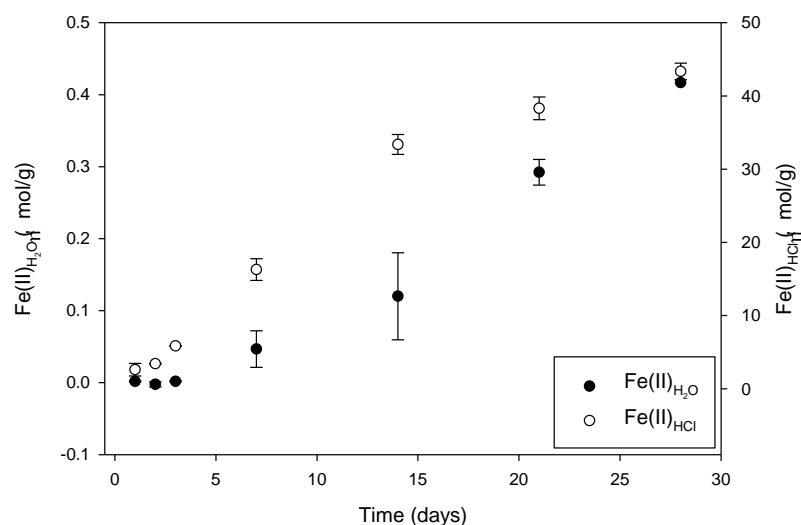


Figure 3.3: Water and HCl extractable iron(II) concentrations under hydrogen amended anoxic conditions during preincubation (error is standard deviation of mean data point).

Sulfate concentration was initially high, then decreased by 7 days and remained very low through the remainder of the preincubation (Figure 3.4). Sulfate reduction occurred fairly quickly. The control and ferrozine amended experiments reached very low concentrations after approximately 14 days, but this faster reduction of sulfate may be due to the presence of hydrogen. Hydrogen is acting as an additional electron donor, causing faster reduction of electron acceptors present in the slurry.

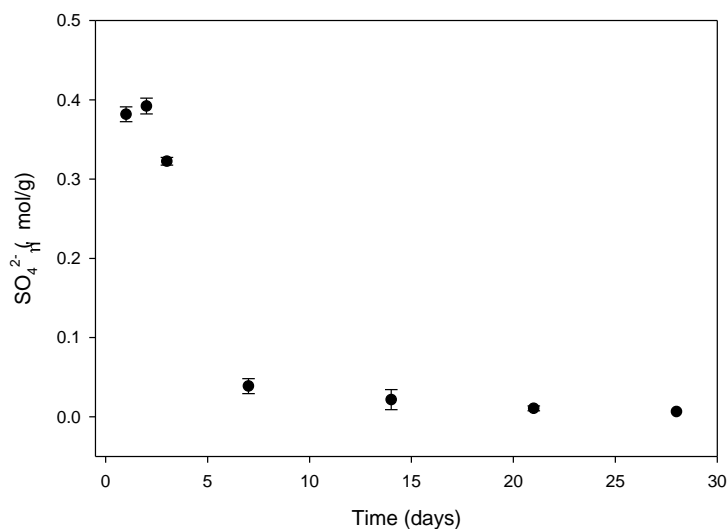


Figure 3.4: Sulfate concentration under hydrogen amended conditions during preincubation (error is standard deviation of mean data point).

In the previous experiments, acetate concentrations would reach $\sim 12 \mu\text{mol g}^{-1}$ after 21 days, and then drop off to $\sim 8 \mu\text{mol g}^{-1}$ by the 28th day. However, in this hydrogen-amended experiment, acetate rose consistently during preincubation, from approximately $3 \mu\text{mol g}^{-1}$ to about $13 \mu\text{mol g}^{-1}$ (Figure 3.5). Where methanogens may have been utilizing acetate at later times during preincubation in the control and ferrozine experiments, resulting in the drop in acetate concentration at 28 days, it appears that fermentation was continuously occurring during preincubation, as the acetate concentration never dropped. Some fermentations result in the production of acetate (Paul and Beauchamp, 1989).

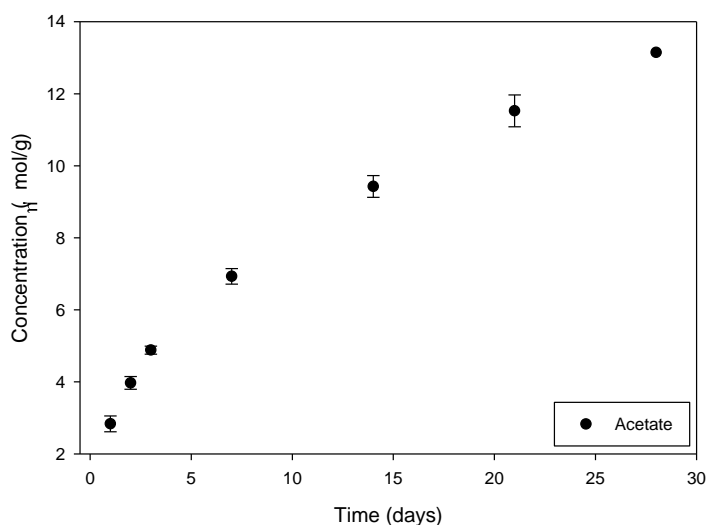


Figure 3.5: Acetate concentration under hydrogen amended anoxic conditions during preincubation (error is standard deviation of mean data point).

Post Nitrate Addition

After adding NaNO_3 , nitrate concentration fell over the first 6 hours at a rate of $0.05 \mu\text{mol g}^{-1} \text{ h}^{-1}$ (Figure 3.6). This is a higher rate of reduction than in the control experiment (Figure 2.6). A higher rate of reduction could be due to competition among nitrate-reducing bacteria, either heterotrophs or lithotrophs, for hydrogen. Nitrate reducers have been shown to efficiently utilize H_2 , which would explain the faster nitrate reduction in the hydrogen-amended experiments (Chidthaisong and Conrad, 2000). In fact, the presence of nitrate lowers H_2 concentrations more than any other TEA (Hoehler et al., 1998). Nitrite concentration remained consistent near the detection limit over the first 6 hours following nitrate addition (Figure 3.6). After 24 hours, there was a very slight increase in the

concentration of nitrite, but the concentration was still very low, at $0.1 \mu\text{mol g}^{-1}$. During the first 6 hours after nitrate addition there was also a rise in nitrous oxide emission (Figure 3.7). However, after 24 hours, nitrous oxide production stopped. The presence of nitrous oxide over the first 6 hours, while nitrate concentration was decreasing, indicates N_2O was present as an intermediate during the reduction of nitrate. After nitrate was fully reduced, the formation of nitrous oxide was no longer seen.

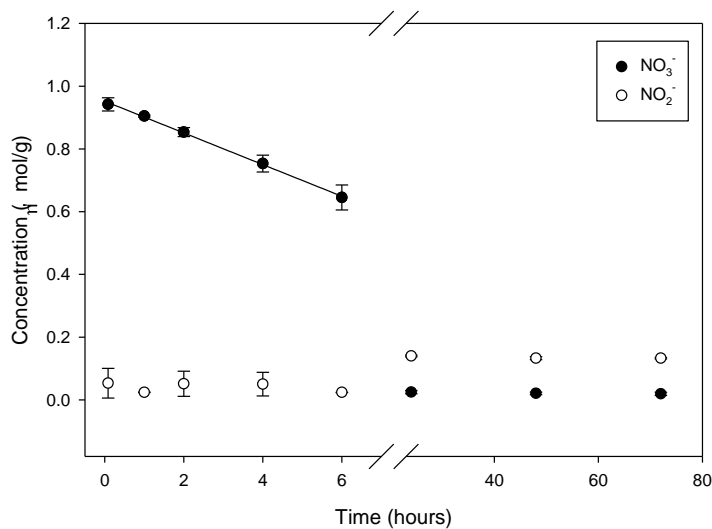


Figure 3.6: Hydrogen amended soil nitrate and nitrite concentrations after nitrate addition under anoxic conditions. ($y = -0.051x + 0.9521$; $R^2 = 0.999$; error is standard deviation of mean data point).

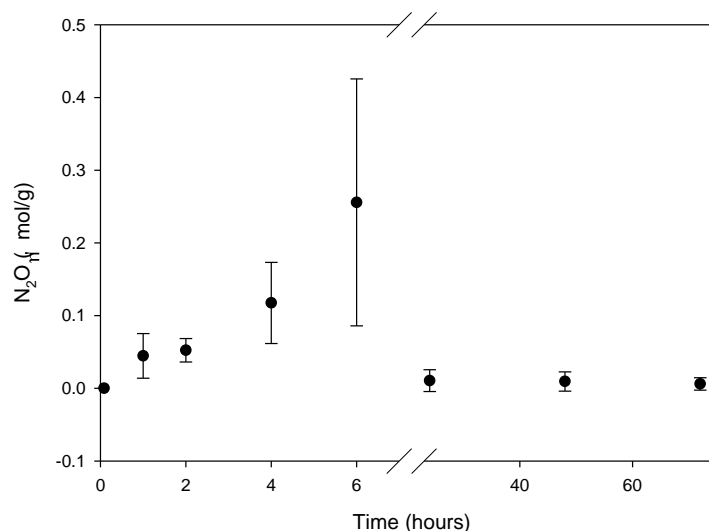


Figure 3.7: Hydrogen amended soil nitrous oxide concentration after nitrate addition under anoxic conditions (error is standard deviation of mean data point).

Water extractable manganese(II) oxidized rapidly after the nitrate addition (Figure 3.8). The concentration dropped from $\sim 1.4 \mu\text{mol g}^{-1}$ to $\sim 0.7 \mu\text{mol g}^{-1}$ over the 72 h period. The initial rate of Mn(II) oxidation during the first 6 h was $0.062 \mu\text{mol g}^{-1} \text{ hour}^{-1}$. The concentration of acid extractable manganese(II) varied with time and showed no clear pattern. While the rate of nitrate reduction was faster with the addition of hydrogen, the rate of oxidation of Mn(II) after nitrate addition was slower than in the control experiment (Figure 2.9, $-0.178 \mu\text{mol g}^{-1} \text{ hour}^{-1}$). This might be explained by competition between H_2 and Mn(II) as electron donors. Hydrogen may have acted as the predominant nitrate reductant, causing a slower rate of Mn(II) oxidation (Lovley and Goodwin, 1988). Hydrogen is a good substrate for nitrate-reducers (Chidthaisong and Conrad, 2000; Strohm et al., 2007). Another explanation might be that the high rate of Mn(II) oxidation in the control was due to a combination of nitrate reduction and adsorption reactions with freshly formed Fe(III) oxide minerals (Junta and Hochella, 1994). Addition of ferrozine allowed isolation of the Mn(II) contribution to nitrate reduction by complexing Fe(II) in the control. In the hydrogen-amended experiments, we did not add ferrozine to isolate the impact of Mn(II). Nonetheless, an estimate of the contribution of Mn(II) to nitrate reduction using rates in Figures 3.6 and 3.8 showed that Mn(II) oxidation accounted for 50% of the reduced nitrate

($0.0248/0.050=0.496$). This is roughly double that of the control and should be regarded as an overestimate pending additional experiments with ferrozine.

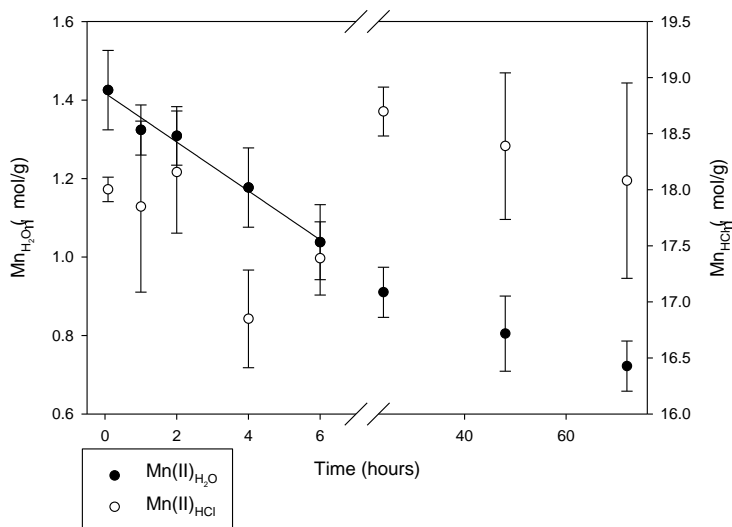


Figure 3.8: Hydrogen amended soil water and HCl extractable Mn(II) concentrations post nitrate addition (Water extractable Mn(II) = $-0.0623x + 1.42$; $R^2 = 0.9831$; error is standard deviation of mean data point).

Water extractable iron(II) exhibited a trend similar to that of the water extractable manganese(II). After adding nitrate, there was a rapid drop in the concentration of water extractable Fe(II) at a rate of $-0.025\mu\text{mol g}^{-1} \text{ hour}^{-1}$ (Figure 3.9). This rate of Fe(II) oxidation is slower than in the control (Figure 2.10, $-0.055\mu\text{mol g}^{-1} \text{ hour}^{-1}$). As with the oxidation of Mn(II), the slower rate could be due to preferential reduction of nitrate with hydrogen as the electron donor (Achnich et al., 1995). Acid extractable Fe(II) remained constant after the addition of nitrate (Figure 3.9). Fe(II) oxidation accounted for 10% of the nitrate reduced ($.005/.05=.10$).

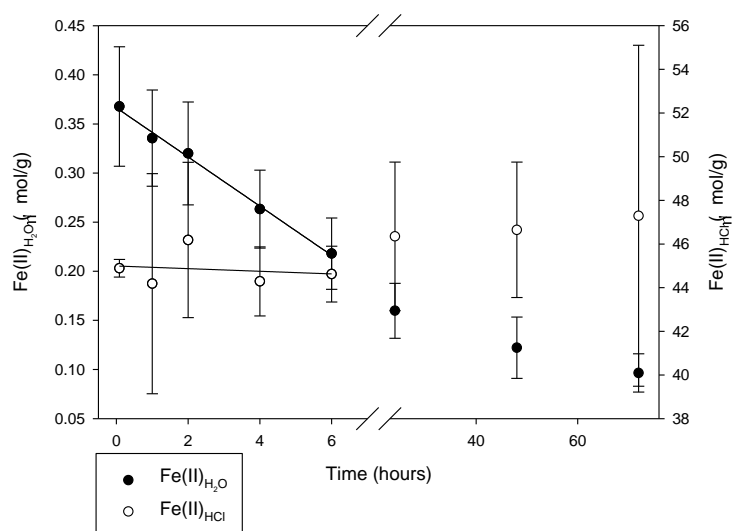


Figure 3.9: Hydrogen amended soil water and HCl extractable Fe(II) concentrations post nitrate addition (Water extractable Fe(II) = $-0.025x + 0.366$; $R^2 = 0.995$ and HCl extractable Fe(II) = $-0.06x + 44.99$; $R^2 = 0.032$; error is standard deviation of mean data point).

Sulfate concentration was variable after nitrate was added under the hydrogen-amended conditions (Figure 3.10). Concentration of sulfate remained low and only showed a distinct increase at 72 hours after the addition of nitrate.

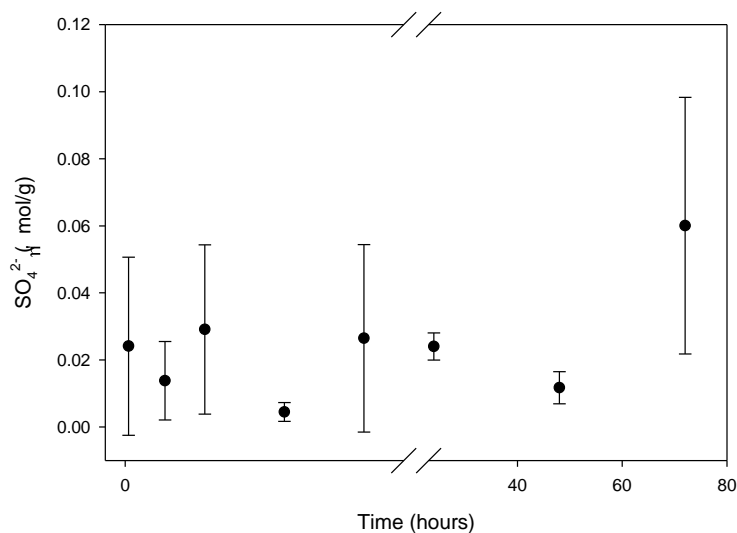


Figure 3.10: Sulfate concentration after nitrate addition under hydrogen amended conditions (error is standard deviation of mean data point).

The concentration of acetate remained fairly constant over the 72 hours after nitrate was added to the soil slurries (Figure 3.11). This lack of change in acetate concentration could be explained by inhibition of processes like methanogenesis via toxic intermediates of denitrification as described in Chidthaisong and Conrad (2000). While acetate remained constant, total DIC increased over the 72 hours after nitrate addition (Figure 3.12). Total cumulative DIC rose from $\sim 8 \mu\text{mol g}^{-1}$ to $\sim 21 \mu\text{mol g}^{-1}$. Increase in CO_2 has been attributed to the conversion of acetate and H_2 to CO_2 when inorganic electron acceptors are available (Chidthaisong and Conrad, 2000). However, because there is a rise in DIC after nitrate addition, but no decrease in acetate concentration, this may indicate that microorganisms are utilizing a different carbon source or hydrogen itself in the increased production of DIC.

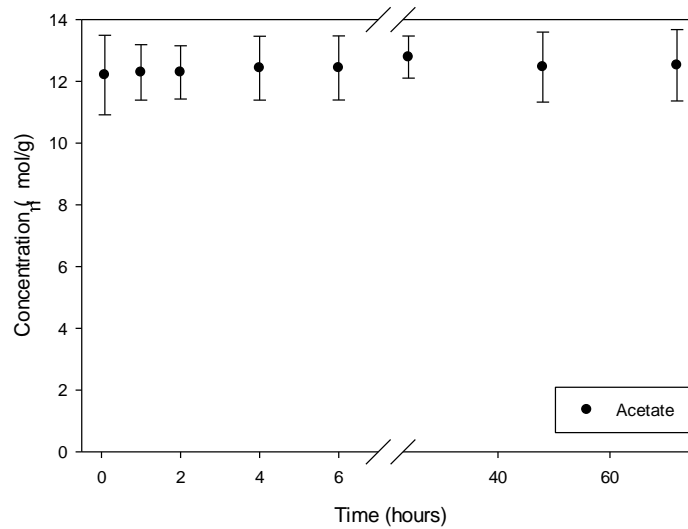


Figure 3.11: Acetate concentration after nitrate addition under hydrogen amended anoxic conditions (error is standard deviation of mean data point).

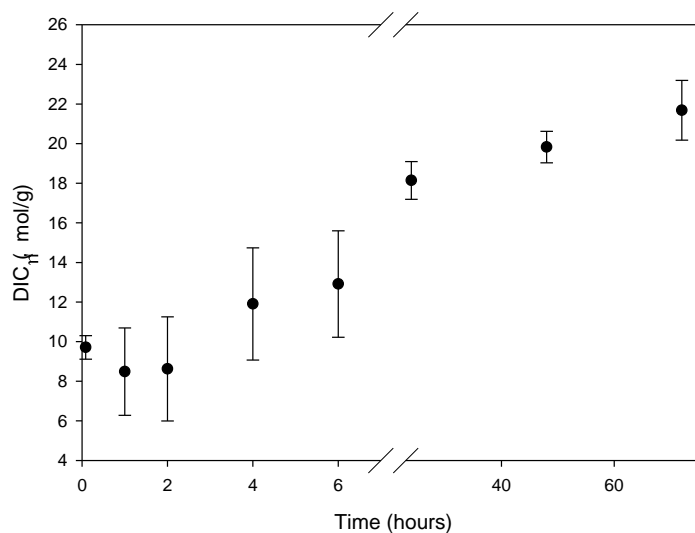


Figure 3.12: Total cumulative dissolved inorganic carbon (DIC) after nitrate addition under hydrogen amended anoxic conditions (error is standard deviation of mean data point).

After adding nitrate, the soil pH increased consistently from 7.75 to a pH of ~ 8.5 (Figure 3.13). This final pH of ~ 8.5 is much higher than in the control (Figure 2.8) or ferrozine amended (Figure 2.19) experiment, which is consistent with the high levels of DIC seen in Figure 3.12. Redox potential after nitrate addition experienced a slight drop initially over the first six hours from approximately -150mV to $\sim 165\text{mV}$ (Figure 3.13). After 24 hours, the redox potential rose to $\sim 130\text{mV}$. This final Eh value is more negative than in the control (Figure 2.8) or ferrozine amended (Figure 2.19) experiments.

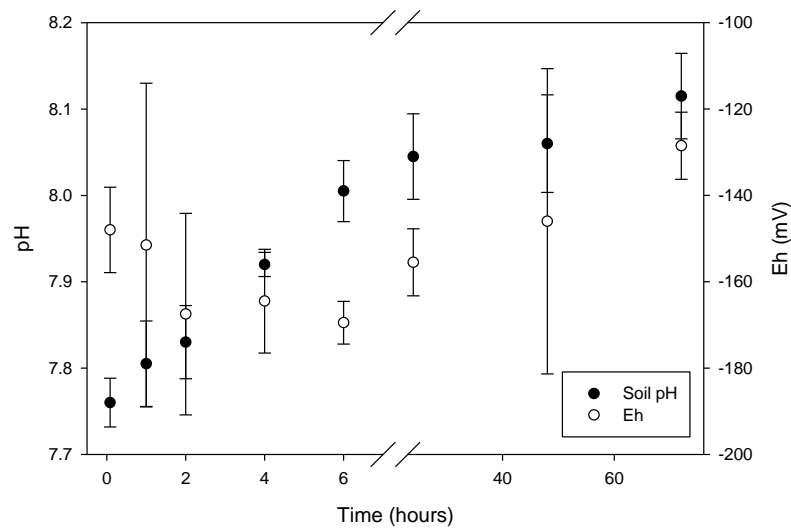


Figure 3.13: Soil pH and Eh, after nitrate addition, under hydrogen amended anoxic conditions (error is standard deviation of mean data point).

Plant Residue Amendment

Preincubation

During preincubation both the high C:N (low nitrogen - LN) amended and the low C:N (high nitrogen - HN) amended slurries had similar trends. In both the LN and HN amended slurries, the soil pH started at ~ 7.35 , rose over the first 3 days to between pH 7.4 and 7.45, then dropped to approximately 7.38 for the remainder of the incubation (Figure 3.14). There were also similar trends in the redox potential of LN and HN treated soil slurries. The Eh for the HN treatment started at ~ 0 mV while the LN treatment started at approximately -10 mV (Figure 3.14). Both treatments saw a drop in Eh to -100 mV over the first 7 days, then an increase and plateau at approximately -50 mV for the remainder of the preincubation (Figure 3.14). Initial pH was lower than the control's initial pH (Figure 2.1) because of the addition of the plant residue. Decomposing organic matter, such as the added wheat residue, produces hydrogen ions, which would result in a lower initial pH (Havlin et al., 2005). However, the gradual increase in pH may be due to oxidation of additional low molecular weight organic acids, like formate, present via the residue amendment, in coordination with iron reduction resulting in a consumption of protons (Salas et al., 2009).

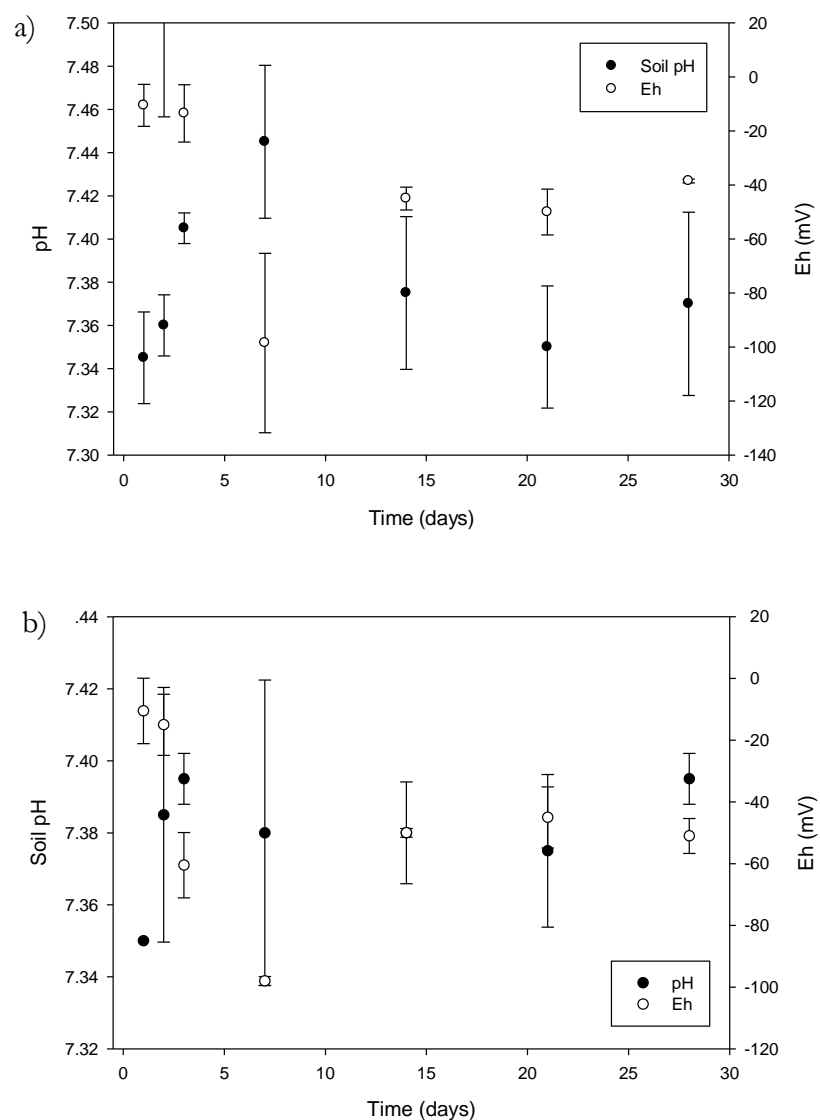


Figure 3.14: pH and Eh during preincubation of high nitrogen (HN) (a) and low nitrogen (LN) (b) residue amended soil slurries (error is standard deviation of mean data point).

During preincubation native manganese was reduced to Mn(II). This process was more rapid in the HN and LN treated experiments than in the control (Figure 2.2). The plateau in water extractable Mn(II) occurs at day 21 in both the HN and LN treatments (Figure 3.15 a,b). In addition, the rate of reduction was faster than in all other treatments. This faster rate was expected because when plant residues are added to the soil slurry, the time it takes for reduction of oxidant decreases drastically due to the ratio of electron donors to electron acceptors (Kumaraswamy et al., 2001).

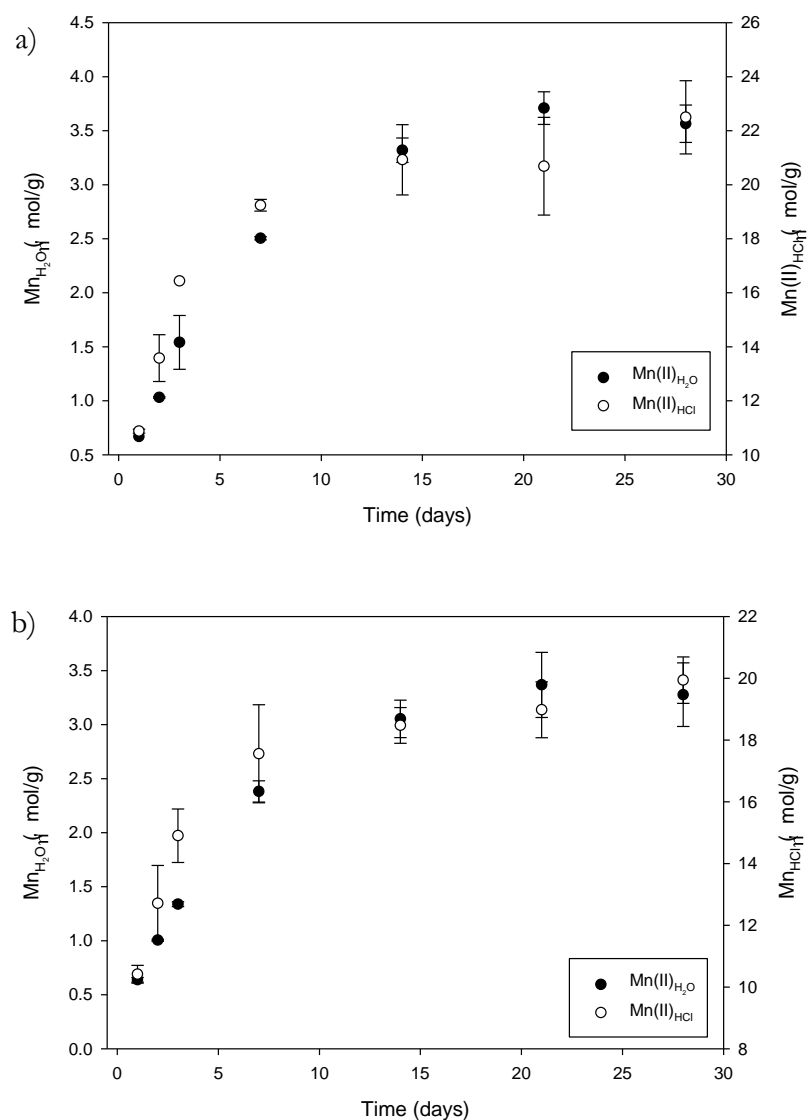


Figure 3.15: Water and acid extractable Mn(II) concentrations during preincubation of high nitrogen (HN) (a) and low nitrogen (LN) (b) residue amended soil slurries (error is standard deviation of mean data point).

Both HN and LN residue amended slurries showed the same trend in the reduction of iron(III) during preincubation (Figure 3.16). Water extractable Fe(II) concentration rose from almost $0\mu\text{mol g}^{-1}$ to $1.1\mu\text{mol g}^{-1}$ while the concentration of acid extractable Fe(II) rose from $0\mu\text{mol g}^{-1}$ to $50\mu\text{mol g}^{-1}$ in both the HN and LN treatments (Figure 3.16 a,b). Reduction of Fe(III) in the water extractable fraction was more rapid than in any other treatment. This is supported by the results of Liptzin and Silver (2009), which gave evidence that rates of Fe(III) reduction increased with the amount of carbon added to the soil.

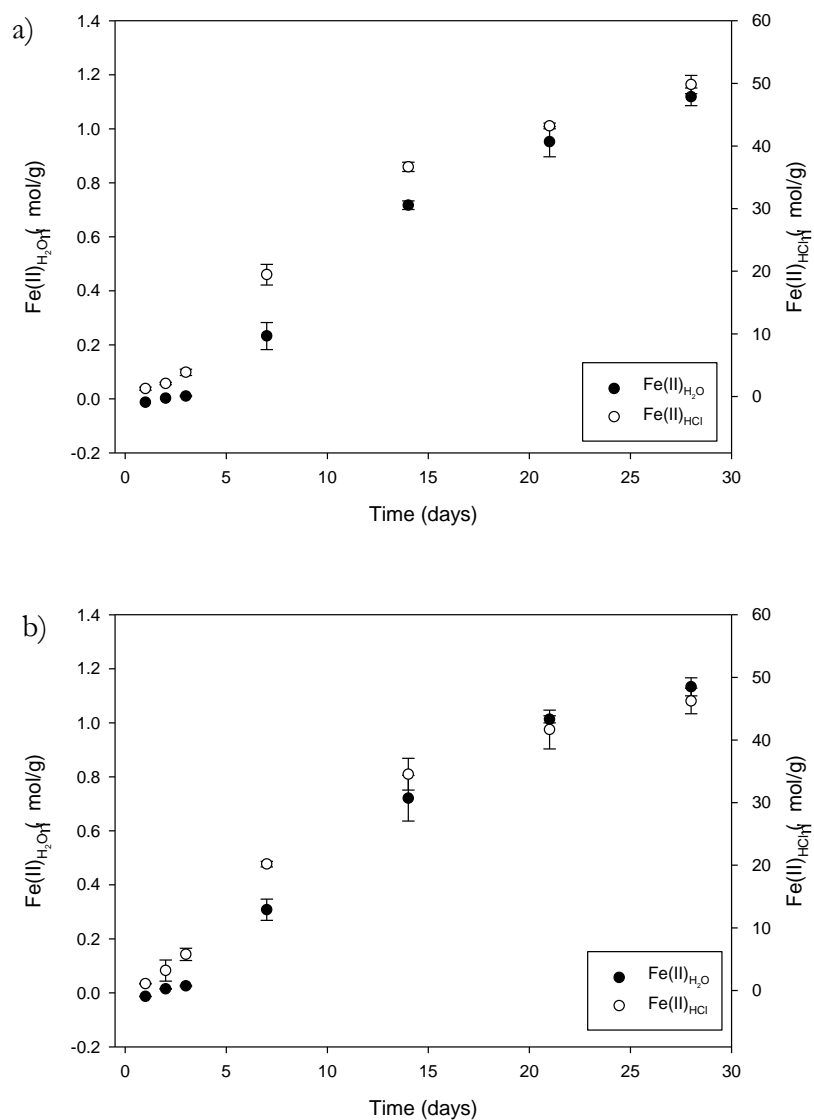


Figure 3.16: Water and HCl extractable Fe(II) concentrations during preincubation of high nitrogen (HN) (a) and low nitrogen (LN) (b) residue amended soil slurries (error is standard deviation of mean data point).

Concentrations of sulfate in both HN and LN residue amended slurries were very similar. The sulfate concentration started high and then dropped after 7 days (Figure 3.17 a,b).

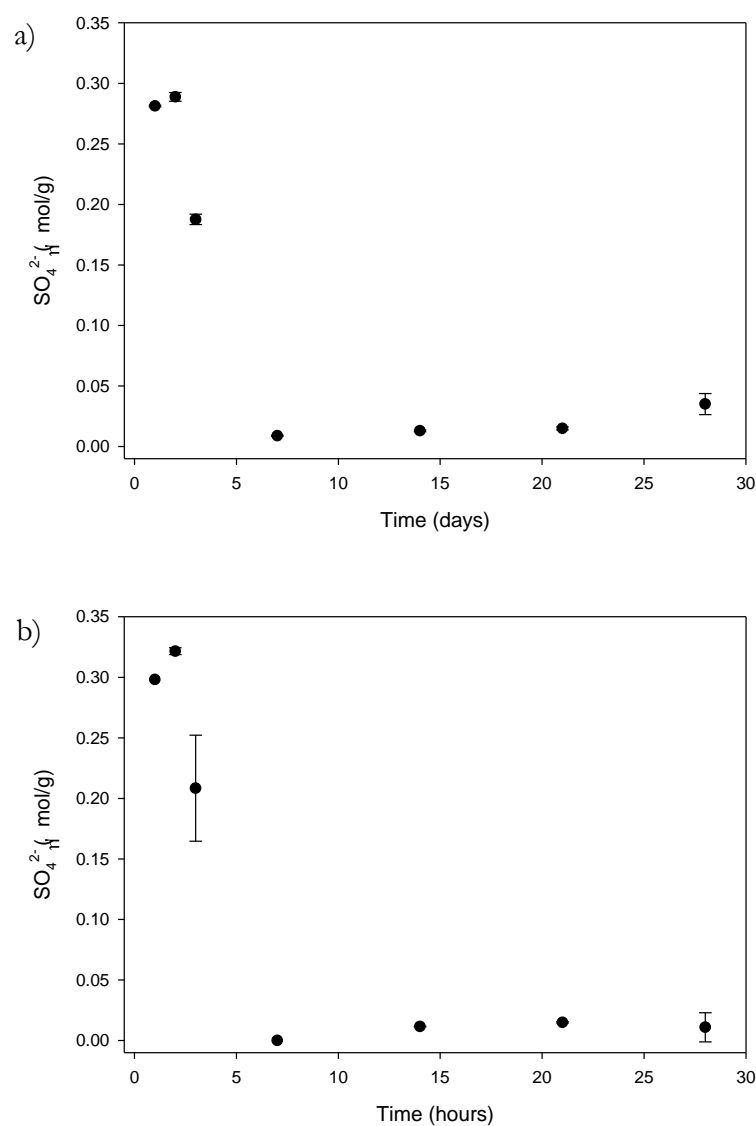


Figure 3.17: Sulfate concentrations in the high nitrogen (HN) (a) and low nitrogen (LN) (b) residue amended soil slurries during preincubation (error is standard deviation of mean data point).

Acetate concentrations in both HN and LN residue amended slurries rose over the first 21 days, and then greatly decreased by day 28 of preincubation (Figure 3.18 a,b). The acetate concentration rose to about $20 \mu\text{mol g}^{-1}$ by day 21, which was a higher peak concentration of acetate than in all other treatments. This greater production of acetate may be a result of the wheat residue addition. The addition of plant residue has been shown to stimulate acetate production via fermentation by microbes (Conrad and Klose, 2011). The

drastic drop in acetate concentration at day 28 may be due to the end of fermentation as consumption of acetate begins via acetoclastic methanogenesis (Conrad and Klose, 2011).

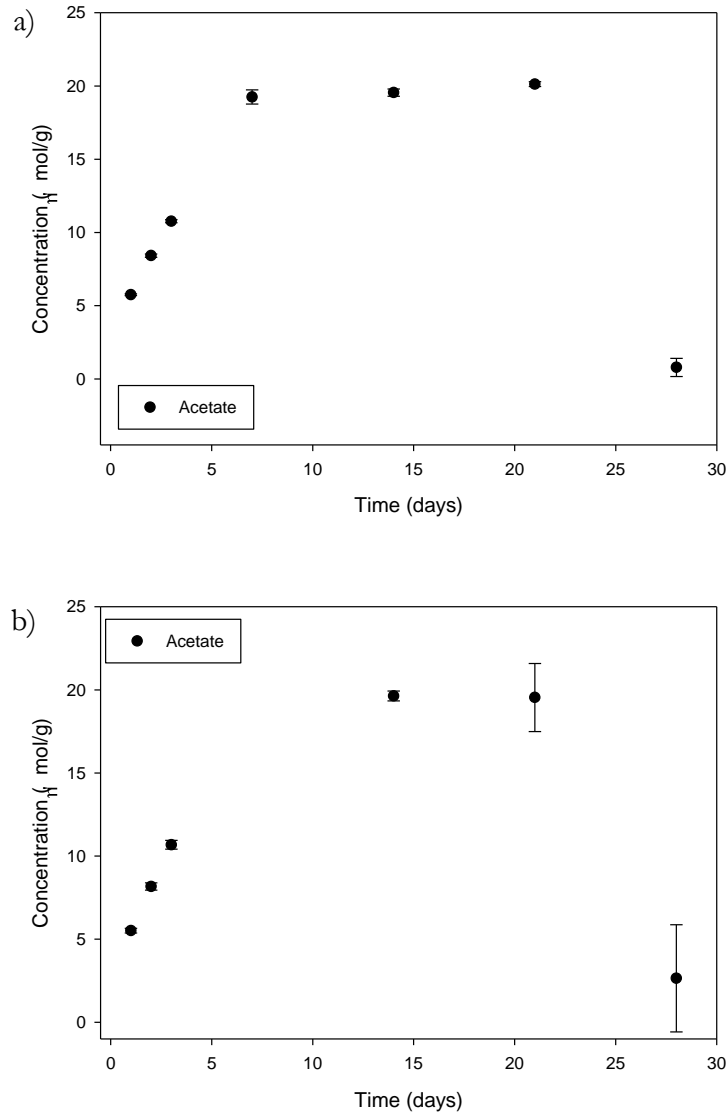


Figure 3.18: Acetate concentrations during preincubation of the high nitrogen (HN) (a) and low nitrogen (LN) (b) residue amended soil slurries (error is standard deviation of mean data point).

Post Nitrate Addition

Nitrate reduced over the first six hours after NaNO_3^- addition at a rate of $0.030 \mu\text{mol g}^{-1} \text{h}^{-1}$ for the HN treated slurries and a rate of $0.031 \mu\text{mol g}^{-1} \text{h}^{-1}$ for the LN treated slurries (Figure 3.19 a,b). These rates are similar to the rate of nitrate reduction in the control

experiment, which was $0.027 \mu\text{mol g}^{-1} \text{h}^{-1}$. From this, it appears that the addition of HN and LN plant residue had a minimal effect on the rate of nitrate reduction under anaerobic conditions. This contradicts studies that have shown the denitrification capacity is increased with an increase in total organic carbon and water-soluble organic carbon or additions of plant residues (Burford and Bremner, 1975; Beauchamp et al., 1989). There was almost no N_2O production after nitrate addition (Figure 3.20). This is an interesting result because the nitrate concentration is falling, but there was no intermediate production of nitrous oxide, a result that was different from all the other treatments. However, nitrite is present as an intermediate in both HN and LN treated soil slurries (Figure 3.19 a,b). There was a much greater level of nitrite in the LN treated soil slurry. A lack of nitrous oxide production may indicate that nitrate is transforming quickly to N_2 because ammonium was not produced and immobilization is unlikely.

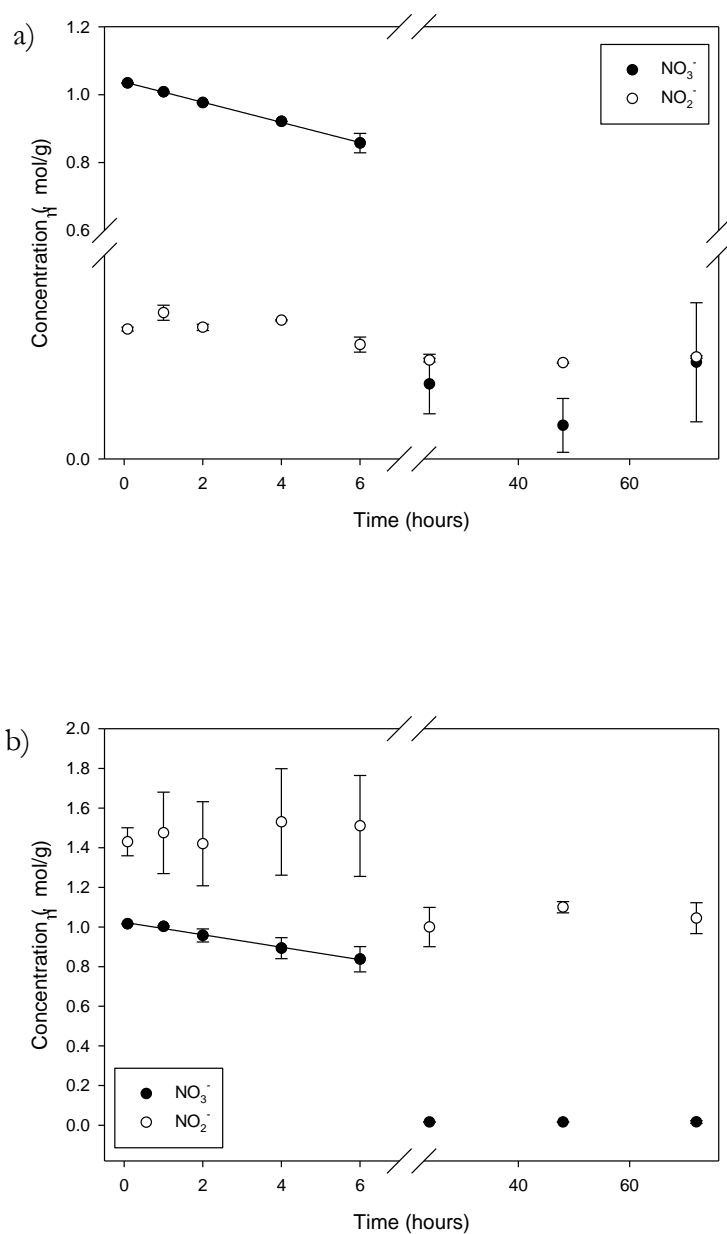


Figure 3.19: Nitrate concentration after NaNO_3 addition in high nitrogen (HN) (a) and low nitrogen (LN) (b) residue amended soil slurries. (HN: $y = -0.030x + 1.037$; $R^2 = 0.999$ and LN: $y = -0.031x + 1.023$; $R^2 = 0.992$; the error is standard deviation of mean data point).

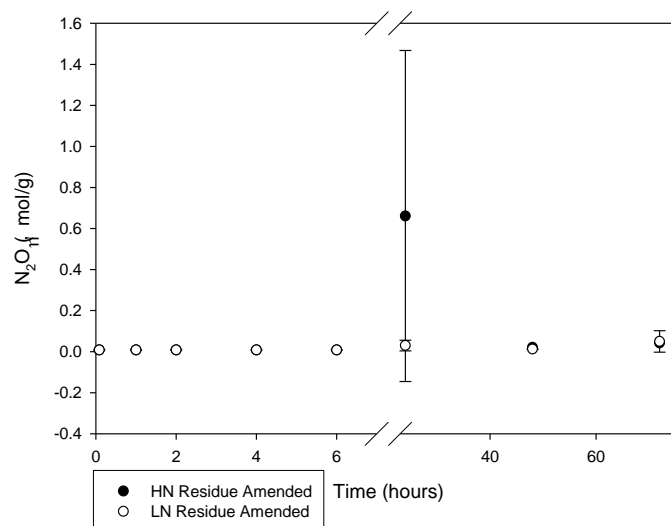


Figure 3.20: Nitrous oxide production after nitrate addition in high nitrogen (HN) and low nitrogen (LN) residue amended slurries (error is standard deviation of mean data point).

Water extractable Mn(II) decreased rapidly over the first 6 hours after nitrate was added. The rate of disappearance of water extractable Mn(II) was $0.11\mu\text{mol g}^{-1} \text{ h}^{-1}$ in the HN amended treatment and $0.114\mu\text{mol g}^{-1} \text{ h}^{-1}$ in the LN amended treatment (Figure 3.21 a,b). Compared to the control experiment, $0.18\mu\text{mol g}^{-1} \text{ h}^{-1}$, the oxidation of Mn(II) in both HN and LN residue amended experiments was much slower. The oxidation of Mn(II) is expected with the addition of plant residue because Mn(II) acted as an electron donor to enhance nitrate reduction, therefore promoting oxidation of Mn(II) once the NaNO_3 was added. An estimate of the contribution of Mn(II) to nitrate reduction using rates in Figures 3.19 and 3.21 showed that Mn(II) oxidation accounted for 148% of the nitrate reduced in the HN treatment ($0.044/0.0297=1.48$) and 145% in the LN treatment ($0.0456/.0314=1.45$). These are gross overestimations, which would require corrections via ferrozine-amended experiments.

Given that the water extractable Mn(II) oxidation rate in the ferrozine-treated soil slurry was roughly 20% of the control ($0.036/0.178$, see Chapter 2), we used this to adjust oxidation rates in Figure 3.21 as one way to provide a more reasonable estimate of the contribution of Mn(II) oxidation to nitrate reduction. This approach showed that Mn(II) oxidation contributed 30% to nitrate reduction in the HN treatment and 29% in the LN treatment. Although these numbers need to be firmed up with additional experiments where

ferrozine is added to the preincubated bottles, they do suggest that wheat residue additions also provide contributions of Mn(II) oxidized to nitrate reduced.

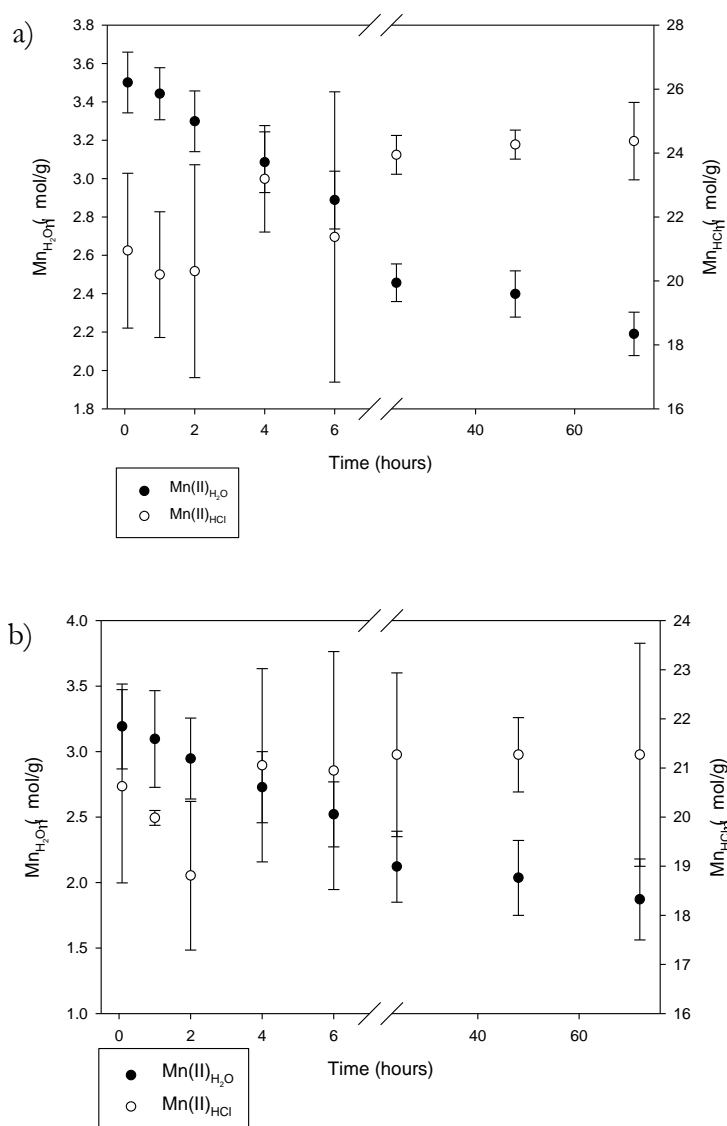


Figure 3.21: Water and HCl extractable Mn(II) concentrations after nitrate addition to the high nitrogen (HN) (a) and the low nitrogen (LN) (b) residue amended soil slurries (HN water extractable Mn(II) = $-0.110x + 3.52$; $R^2 = 0.996$ and LN water extractable Mn(II) = $-0.114x + 3.2$; $R^2 = 0.997$; error is standard deviation of mean data point).

The first 6 hours after the addition of nitrate there was a rapid oxidation of water soluble iron(II) in both the HN and LN treated slurries (Figure 3.22 a,b). The rate of water extractable Fe(II) disappearance was $0.078\mu\text{mol g}^{-1}\text{ hour}^{-1}$ in the HN treatment and $0.087\mu\text{mol g}^{-1}\text{ hour}^{-1}$ in the LN treatment. The rates of Fe(II) oxidation in these residue

amended experiments are faster than the rate of Fe(II) oxidation in the control experiment. In addition, the contribution of Fe(II) oxidation to nitrate reduction in the HN treatment was 53% ($0.0158/.0297=.532$) and 55% in the LN treatment ($0.0174/.0297=.554$). Adjusting these contributions, to account for Fe(II) oxidation by MnO_2 , results in a value of 26% and 27% in the HN and LN treatments. These contributions are higher than in the control and hydrogen treated experiments and support our hypothesis.

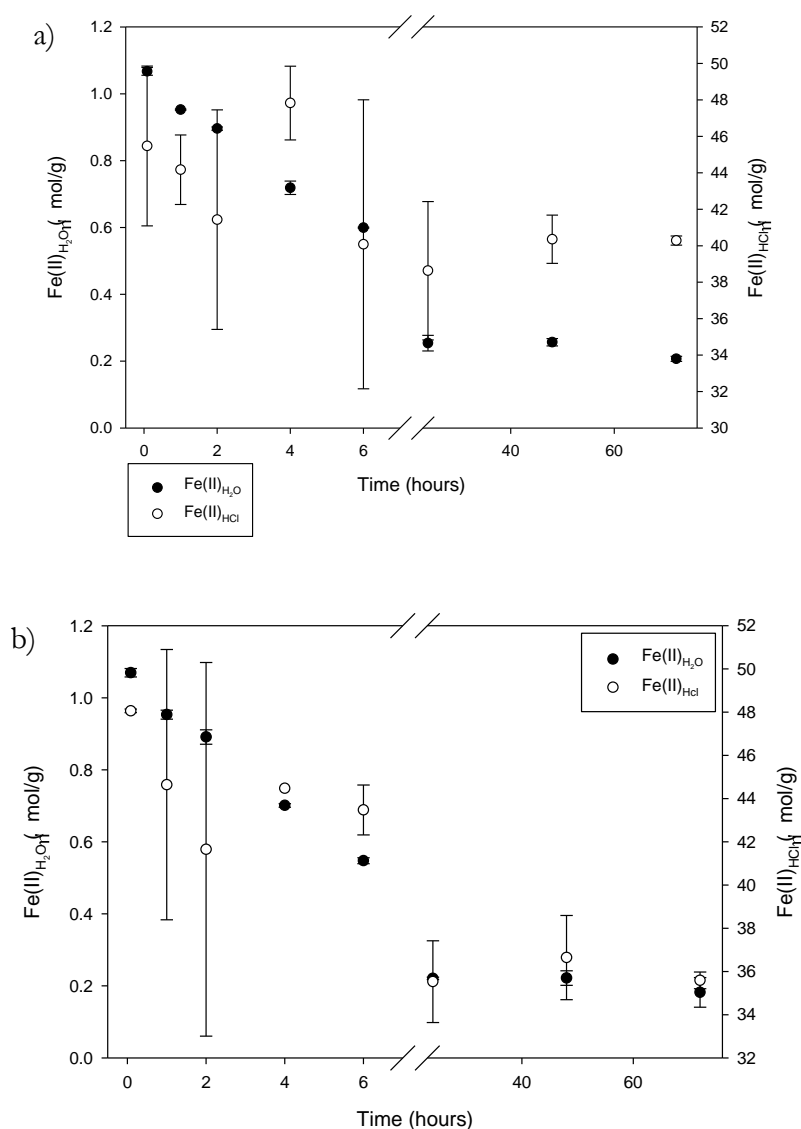


Figure 3.22: Water and HCl extractable Fe(II) concentrations after nitrate addition to high nitrogen (HN) (a) and low nitrogen (LN) (b) residue amended soil slurries (HN water extractable Fe(II) = $-0.078x + 1.05$; $R^2 = 0.988$; LN water extractable Fe(II) = $-0.087x + 1.06$; $R^2 = 0.995$; error is standard deviation of mean data point).

Sulfate concentration varied and had no distinct pattern after the addition of nitrate to either the HN or LN residue amended slurries (Figure 3.23 a,b). However, the concentration of sulfate in the LN amended slurry (Figure 3.23b) is approximately 100-fold higher than in the HN amended slurry (Figure 3.23a).

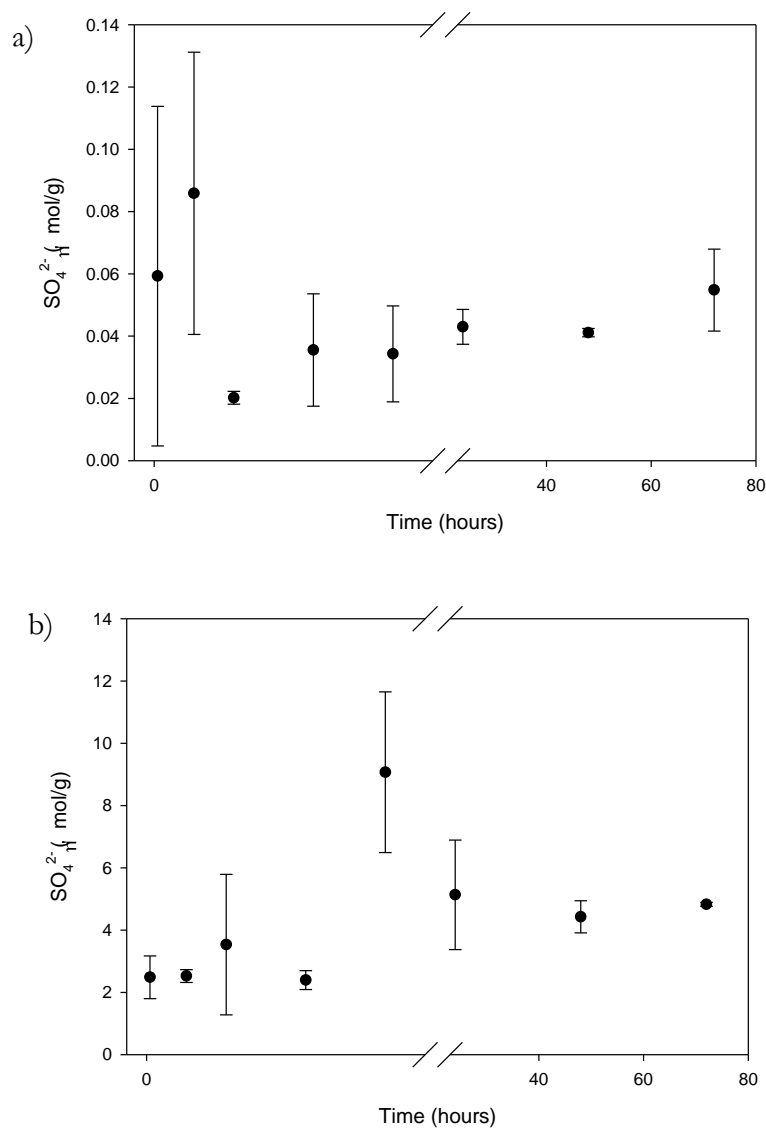


Figure 3.23: Sulfate concentration after nitrate addition in the high nitrogen (HN) (a) and low nitrogen (LN) (b) residue amended soil slurries (error is standard deviation of mean data point).

Levels of acetate, which started very low due to depletion during the preincubation (Figure 3.18), increased continuously over the next 72 hours in the HN residue treated soil slurries after nitrate addition (Figure 3.24a). Acetate concentrations were much lower than in the control and hydrogen amended experiments, but there was still a general increase in acetate concentrations. The increased presence of organic acids from the plant residue could be utilized by denitrifiers, which provides more energy to fermentative bacteria, resulting in increased production of acetate (Beauchamp et al., 1989). The preferred products of

fermentative bacteria are acetate and H_2 (Beauchamp et al., 1989; Paul and Beauchamp, 1989). Extremely high levels of acetate were attained in the LN residue treated soil slurries after nitrate addition (Figure 3.24b). High levels of acetate in the LN treatment suggest the presence of biochemical properties in the residue that were not accounted for, which may have influenced acetate concentrations in this specific treatment.

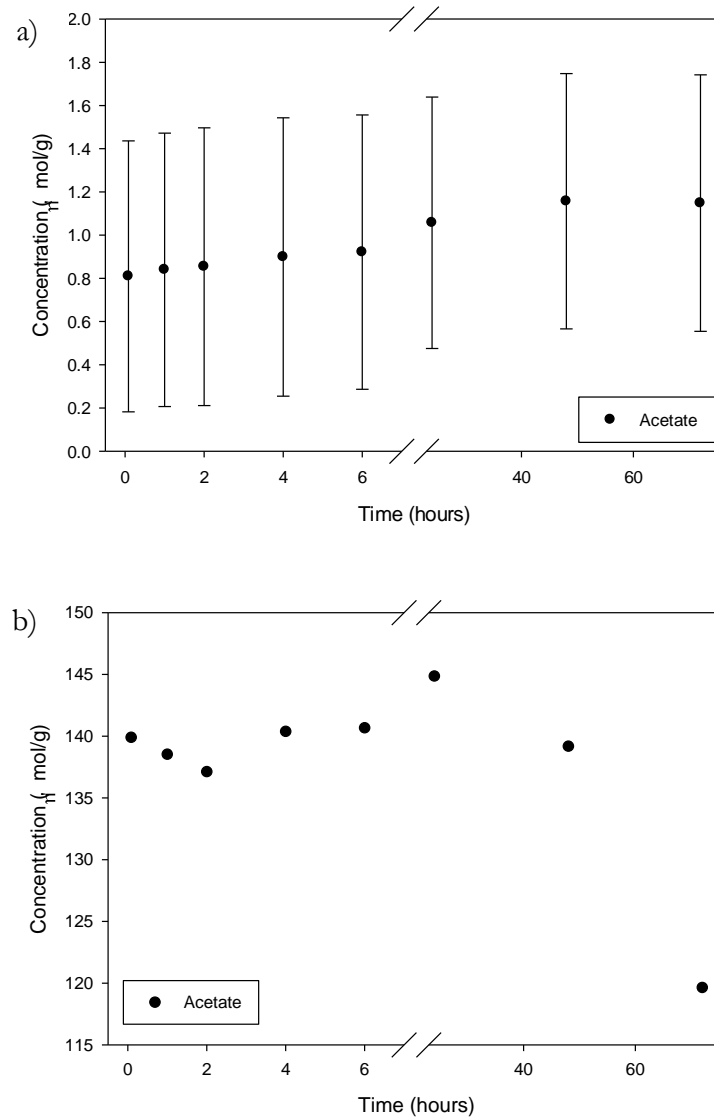


Figure 3.24: Acetate concentrations after nitrate addition to high nitrogen (HN) (a) and low nitrogen (LN) (b) residue amended soil slurries (error is standard deviation of mean data point).

There were slight increases in DIC in the first six hours after nitrate addition to both HN and LN residue amended soil slurries (Figure 3.35). Given that there is still some nitrate

reduction that is unaccounted for (the sum of Mn(II) and Fe(II) oxidation accounted for approximately 56% of the nitrate reduced in these treatments), this increase in DIC might be due to the involvement of heterotrophic denitrifiers (Figure 3.25). However, the large error bars precluded any discussion of treatment differences between HN and LN.

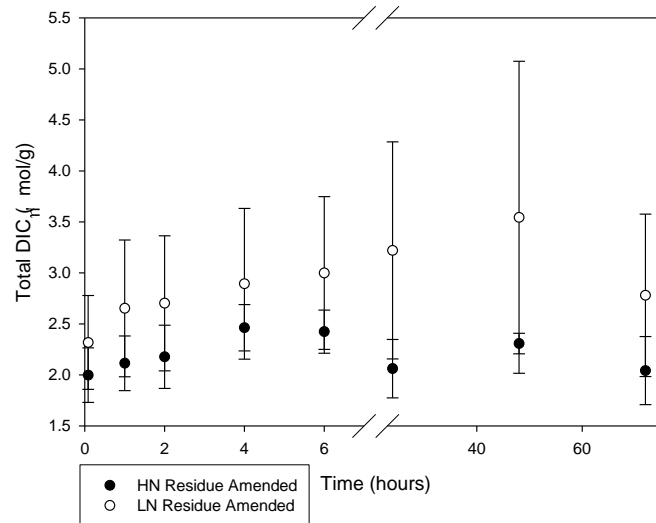


Figure 3.25: Cumulative dissolved inorganic carbon (DIC) after nitrate addition to high nitrogen (HN) and low nitrogen (LN) residue amended soil slurries (error is standard deviation of mean data point).

After nitrate addition, the pH of HN amended slurry rose rapidly over the first six hours, from 7.40 to 7.63 (Figure 3.26 a,b). A similar trend was seen in the LN amended slurry, in which pH rose from 7.44 to 7.66 over the first six hours after nitrate addition (Figure 3.26 a,b). After 24 hours, there was a lower pH plateau in both HN and LN amended slurries, to pH 7.45 and 7.55, respectively. The Eh of both HN and LN amended slurries also showed similar trends. Soil Eh showed an initial drop to -60mV (HN) and -70mV (LN) and then rose gradually over the remaining incubation time, to a final Eh of 20mV (Figure 3.26 a,b).

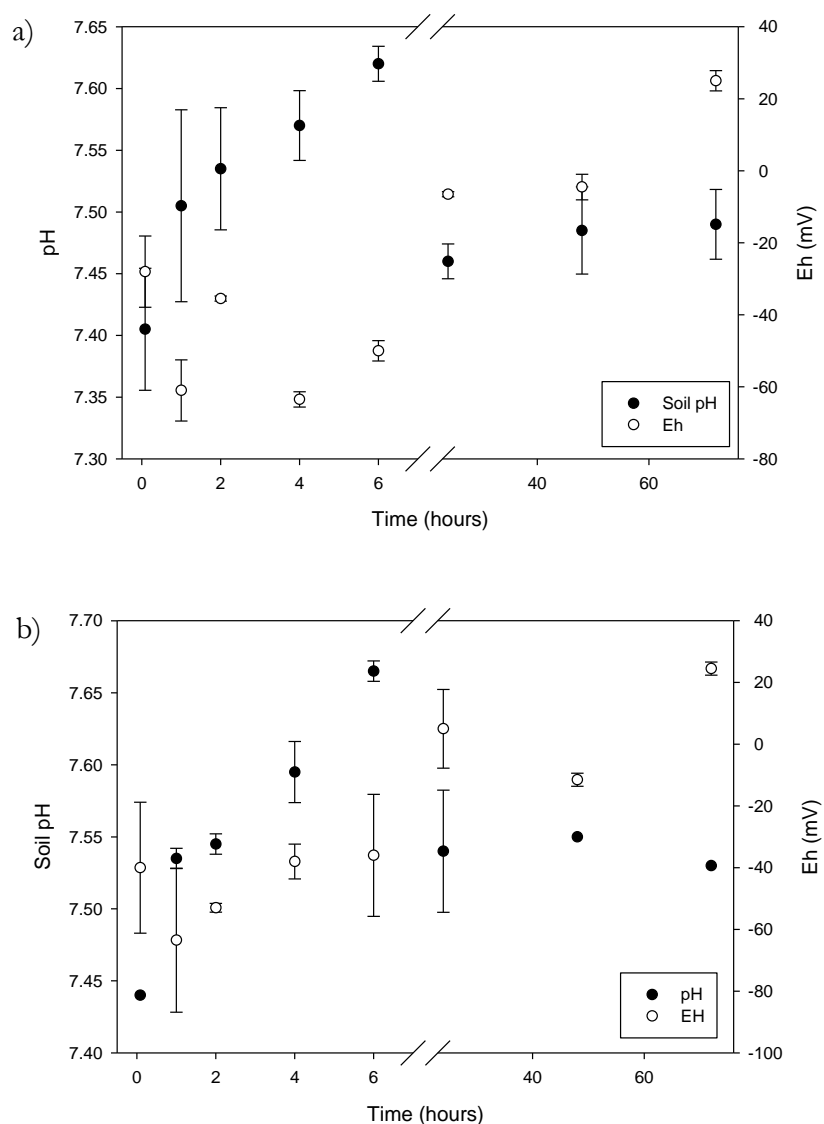


Figure 3.26: Soil pH and Eh after nitrate addition to high nitrogen (HN) (a) and low nitrogen (LN) (b) residue amended soil slurries (error is standard deviation of mean data point).

CONCLUSIONS

The addition of hydrogen gas to the argon atmosphere resulted in an increased reduction of nitrate after the addition of NaNO_3 , faster than the rate of nitrate reduction in the control. The rate of oxidation of Fe(II) and Mn(II) after nitrate addition was slower than in the control experiments. During preincubation, the addition of hydrogen resulted in greater quantities and faster rates of reduction for Fe(III) and Mn(III, IV). The hydrogen

amendment had the greatest effect on species during preincubation, but encouraged a faster rate of nitrate reduction after the addition of nitrate.

Wheat residue amendments to the Sadler soil slurries resulted in a similar rate of nitrate reduction as in the control after the addition of NaNO_3 . Even with the addition of wheat residue before preincubation, nitrate reduction happened concurrently with both Mn(II) and Fe(II) oxidation. The oxidation of Mn(II) and Fe(II) occurred at faster rates than in both the control and hydrogen amended experiments. When plant residue is left on the soil surface, as in no-till agriculture, the addition of nitrate under water-logged conditions could help promote faster oxidation of Mn(II) and Fe(II) .

CHAPTER 4: THESIS CONCLUSIONS

Nitrate is an important component of the soil environment. It is quickly transformed, depending on soil conditions. Quick transformation of nitrate can result in the loss of fertilizer nitrate additions via leaching or, under anaerobic conditions, reduction to gaseous products that pose threats to the atmosphere. Furthermore, abundant iron concentrations in the Earth's crust have been shown to interact with nitrate in the soil. Some literature has also presented a connection between manganese, a less abundant element, and nitrate in soil environments.

The first objective of this study was to establish the contribution of Fe(II) oxidation and Mn(II) oxidation to nitrate reduction. The respective contributions were 39.8% and 25.3% for the first six hours of the reaction. The Fe(II) contribution was lowered to 19% when accounting for the Fe(II)-MnO₂ secondary reaction. These findings are significant in light of the excess of native soil organic carbon over total Fe and Mn, yet, these latter two elements are intimately associated with nitrate reduction. Organic carbon becomes involved in nitrate reduction at longer time periods (>6h) based on the relative increases in dissolved inorganic carbon coupled with acetate depletion. Furthermore, although manganese was 20-fold less abundant than iron in the Sadler soil, its oxidation accounted for a significant portion of nitrate reduction.

The second objective of this study was to examine the effects of hydrogen gas and wheat residue additives as electron donors on the process of nitrate-dependent Fe(II) and Mn(II) oxidation. The addition of hydrogen resulted in the acceleration of nitrate reduction, but decreased the rate of Fe(II) and Mn(II) oxidation. With hydrogen addition, the contribution of Fe(II) oxidation to nitrate reduction was 10%, while the contribution of Mn(II) oxidation to nitrate reduction measured 50%, a much higher value than found in the control experiment, although the Mn(II) oxidation contribution was not corrected. Wheat residue addition had minimal effect on nitrate reduction (post NaNO₃ addition), compared to the control. However, residue addition did result in a significantly faster reduction of Fe(III) during preincubation. Iron(II) oxidation accounted for 53% of nitrate reduction in the HN residue amended experiment and 55% in the LN residue amended experiment. Contributions of Mn(II) oxidation to nitrate reduction were calculated and found to be

overestimations, 148% and 145% in the HN and LN treatments respectively. In the future, experiments adding ferrozine (as in Chapter 2) for hydrogen gas and wheat residue amended experiments would provide more accurate estimates of the contributions of both Fe(II) and Mn(II) oxidation to nitrate reduction.

With the results of these experiments, it has been shown that adding nitrate induces Fe(II) and Mn(II) oxidation. The contributions of Fe(II) and Mn(II) oxidation to nitrate reduction could be implemented in water-logged soil environments where anoxic conditions are prominent, such as rice paddies. When nitrate is added to these flooded soils, it would promote oxidative conditions within the soil to allow for uptake of nutrients. This has potential to reduce the necessity of draining paddy fields.

Future experiments could characterize the active microbes in the processes of nitrate dependent iron(II) and manganese(II) oxidation. More specifically, a focus should be placed on manganese. As mentioned previously, manganese plays a role in the reduction of nitrate under the anaerobic conditions, as demonstrated by the ferrozine treated experiment in chapter 2. Because Mn provides a contribution to nitrate reduction, while being 20-fold less abundant in the soil than Fe, Mn should not be overlooked in future experiments. The use of manganese by active microbes such as *Geobacter* and *Shewanella* may be the mechanism by which these contributions are made (Thamdrup, 2000). Using microbiology techniques such as enumeration, characterization, and protein assays of bacteria involved may provide insights into biological contributions to nitrate reduction via manganese oxidation. Contributions such as these would provide insight regarding Mn reaction mechanisms and could also be done for iron.

While soil organic carbon has been the historic index for nitrate reduction in soil, this research has shown that both iron and manganese should be considered. This is especially important given the negative environmental consequences associated with nitrate reduction, and merits further research.

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Dean's List, University of Kentucky 2009-2010

PROFESSIONAL SOCIETIES

Soil Science Society of America
American Society of Agronomy
Crop Science Society of America

PUBLICATIONS AND TEACHING

Matocha, C. J., P. Dhakal, and **S. M. Pyzola**. 2012. The role of abiotic and coupled biotic/abiotic mineral controlled redox processes in nitrate reduction. *Advances in Agronomy* 115: 181-214.

Teaching Assistant, University of Kentucky, Lexington, Kentucky. Lab instructor for PLS 366: Introduction to Soil Science. Spring 2011.